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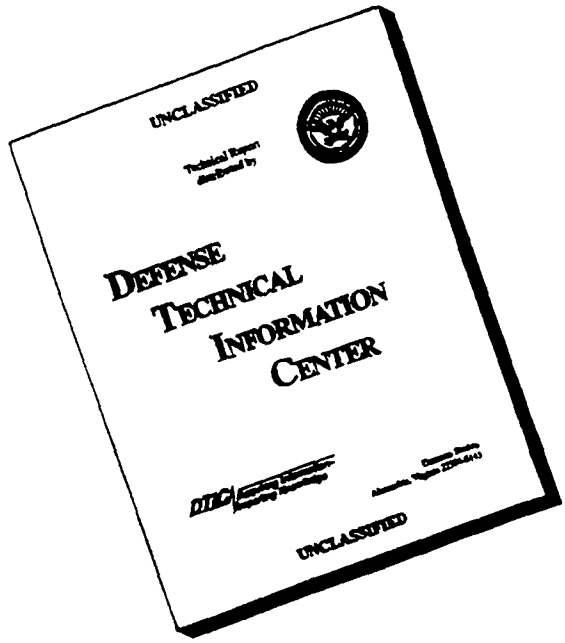

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13. ABSTRACT *(Maximum 200*

Improved methods for frozen storage of glycerolized red blood cells (RBCs) are needed both to meet military and civilian needs in periods of high demand. Existing deglycerolization systems are labor intensive and considered "open" systems resulting in a maximum storage of 24 hours following washing. AHT's thawed blood processing system (TBPS) has been developed through optimization of a disposable configuration and automated hardware console. AHT has met the essential element of the TBPS development plan and demonstrated significant improvement in the quality of blood product washed with the TBPS as well as completed the design, assembly and validation of an automated console to process frozen thawed blood. Key determinants of performance of the TBPS have been determined to be the choice of the flat filter media used and the type of spinning rotor used to mix the blood within the filter. In its final configuration, the TBPS typically yields a washed frozen-thawed blood product with a hematocrit of 60-70%, recovery of 80-90%, and supernatant hgb levels of 150-300mg/dL. The Phase II development has lead to a closed, sterile washing system for thawed blood and should allow for extended storage thawed RBC products pending future incorporation of additive solutions.

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Introduction

The ability to maintain an adequate supply of all types of blood for military and civilian needs in times of high demand must be addressed either by increasing the collection of whole blood units or by making better use of collected blood components. In response to the latter, significant progress has been made in the technology of freezing, thawing and washing red blood cells (RBCs).

The advantages of frozen/thawed (FT) storage of RBCs include the removal of plasma, platelets, white blood cells (WBCs), anticoagulants and the PVC bag plasticizer (DEHP). Cryopreservation also allows for the long-term storage of rare red cell types and selected red cells lacking antigens which commonly sensitize recipients (1). Some reports have indicated that washing of frozen/thawed RBCs (FTRBCs) leads to a reduction in the level of CMV virus (2) through the removal of plasma and white blood cells. In addition, cryopreservation is used for autologous storage when larger volumes of blood are requested for covering planned surgical procedures. Finally, a blood banking system which combines liquid storage and frozen storage of rejuvenated O-positive and O-negative cells would ensure adequate blood supply in times of high demand or emergency. The major disadvantages of frozen RBC storage include limited post-wash storage and higher processing costs, including disposables, hardware and labor costs associated with storage and washing.

This report summarizes the status of the development of a novel, closed sterile system for washing FTRBC using membrane based technology developed by Advanced Haemotechnologies (AHT).

Frozen Storage of RBCs: In the development of FT technology for RBCs, several cryoprotectants have been evaluated for the freezing of RBCs including glycerol, hydroxyethyl starch and polyvinylpyrolidone.

Glycerol is generally accepted as the best cryoprotectant for freezing RBCs. Several factors are known to affect the degree of FT hemolysis of glycerol frozen RBCs, including: red blood cell and glycerol concentration; conditions for mixing blood with glycerol; composition of the freezing container; the freezing rate and storage temperature; and the rate of thawing and thawing temperature (3). Two approaches to freezing RBCs in glycerol have been developed: high glycerol (~40%) with frozen storage at -80°C; and low glycerol (~20%), requiring storage in LN_2 vapor.

The use of 20% glycerol to FTRBCs has yielded good results with respect to RBC recovery and 24 hour in-vivo survival (1,4). This method allows for simpler and faster washing procedures to remove the glycerol. However, the major drawback of this approach is the necessity of maintaining -130°C throughout storage. Studies have shown that increased hemolysis occurs affecting recovery of RBCs if the temperature is elevated above -130°C even for short periods during storage (4). This presents logistical problems with the storage of low glycerol frozen blood in LN_2 vapor, especially during transport.

Freezing RBCs in 40% glycerol simplifies the requirements for freezing and storage, but requires additional washing to effectively remove the glycerol. Cells treated with 40% glycerol are frozen in -80°C freezers in standard PVC bags at a rate of ~1°C/min and stored at this temperature. Transportation of frozen units is simplified through the use of mechanical refrigeration and dry ice to maintain -80°C. Currently, the FDA has approved frozen storage for up to 10 years. Valeri et. al. have reported that FTRBCs can be

thawed and washed after storage for up to 21 years at -80°C and remain safe and therapeutically effective (5).

The intracellular environment of glycerolized cells is hypertonic relative to plasma and the first solution used must be somewhat hypertonic. This allows the glycerol to begin diffusing out of the red cell while the intracellular environment remains hypertonic. After equilibration of the thawed RBCs with a hypertonic solution, the next step is washing with solutions progressively less hypertonic and final suspension in an isotonic electrolyte solution containing glucose. Several approaches to washing thawed cells, previously frozen in 40% glycerol, have been attempted (6,7,8,9). Early methods for deglycerolization required more than 4 L of wash solutions yielding varying recoveries (1). As deglycerolization methods developed, a number of devices, protocols, and wash solutions were evaluated over the past 40 years (6,8,9). With the exception of the agglomeration method developed by Huggins (6,10), where low ionic strength diluents were used to cause reversible agglutination and sedimentation of cells, most methods have relied on centrifuge-based technologies. Today two centrifuge devices for deglycerolization are commonly used: the Cobe 2991 or 2992 and the Haemonetics 115 cell washers. The deglycerolization procedure with both of these devices employs initial dilutions of the thawed blood with 12% sodium chloride followed by 0.9% sodium chloride-0.2% glucose (NaCl-glucose) solutions (1). After dilution, the cells are washed with 1.5 to 2 L of NaCl-glucose with either the Cobe or Haemonetics cell washers. The Cobe cell washer can be operated in manual or automated modes, using a series of batch washing/centrifugation/dilution steps. The Haemonetics cell washer employs a bowl configuration in which the diluted thawed unit is washed continuously with NaCl-glucose. Both the Cobe bag and the Haemonetics bowl have rotary seals making the devices "open systems". Currently, a 24-hour shelf life is imposed by the FDA on previously frozen-thawed, deglycerolized red cells, as well as on non-frozen red cells washed with these existing commercially available cell washers because the rotary seals in these systems are not closed and sterility can be broached during processing (11). With both devices, the washing procedure requires approximately 30 minutes and requires operator intervention during washing.

A major issue regarding the frozen blood supply is the length of time within which RBCs must be used after thawing(12). Sterile docking devices are now available for joining plastic tubing without violating sterility so that the hazard of contamination need no longer be a factor limiting subsequent storage if a washing device with a closed sterile system is available. The AHT Thawed Blood Processing System (TBPS) system is designed to meet this criteria by providing a closed sterile disposable for the washing of RBCs.

The US Department of Defense plans to maintain a stockpile of 300,000 cryopreserved units of RBCs by the year 2004 (4). In order to have these units readily available for use at the battle field site, advances in the washing technology are required, especially with respect to blood sterility and automation. The device proposed for development by AHT will meet both of these requirements. Specifically, this projected device offers several advantages over current centrifuge based technology including: automated dilution and washing protocols; compact, portable hardware; and a sterile, closed washing system allowing for extended storage following deglycerolization.

History of TBPS Development

Cell separation utilizing microporous membrane filters to separate plasma from cells is termed plasma filtration. The first commercialized applications have been therapeutic plasmapheresis and donor plasma collection. Cross flow filtration as opposed to "dead end" filtration has been almost universally used. In recent years, high shear rate designs such as the Baxter Autopheresis - C^R rotating cylinder plasma collection system have proven effective and cost competitive compared to centrifuge systems.

In 1990, L. S. Gordon obtained a patent for "Apparatus and method for the autotransfusion of blood" using a two stage, flat sheet plasma filter. Advanced Haemotechnologies developed a system which made use of membrane based cell separation for intraoperative autotransfusion, the PlateletPlus™Blood Processing System.

Extensive testing performed during the development of the PlateletPlus™ System, a prototype, which demonstrated the suitability of plasma filtration for blood cell washing and concentration in the intraoperative situation. The PlateletPlus™ system advanced the science of cell washing to a new level by combining the three steps of conventional machines (fill, wash and empty) into one continuous process.

The requirements for cell washing for deglycerolization are somewhat different. The PlateletPlus™ System for intraoperative autotransfusion incorporates 2 filter stages to achieve a reduction in supernatant concentration of 75%. It also reduced the supernatant volume by approximately 70%, for a total reduction of 92%. Deglycerolization requires a reduction of supernatant concentration of 98%. However, the total blood volume processed is much lower than typically seen in intraoperative autotransfusion. This suggested that a single stage batch processing system would be more appropriate for deglycerolization. AHT has developed the Thawed Blood Processing System (TBPS) for filtration of frozen-thawed cells during deglycerolization. For this application the filter disposable has been modified into a two membrane, single rotor batch processing device in which a full unit of thawed blood is pumped into the filter housing, concentrated and washed with saline/glucose.

AHT completed the elements of the Phase I proposal with the development of a device which meets the objectives of the Phase I proposal. Specifically, an output hematocrit of approximately 40%, residual glycerol levels of $\leq$ 1% and a unit wash time of less than 25 minutes.

The conclusions of the Phase I experiments were as follows:

- Membrane selection studies indicated that the optimal membrane was a Pall 1.2μ LP membranes. The Pall LP membrane showed the best saline and plasma flow rates. Evaluation in autotransfusion devices indicated the Pall LP membrane was least susceptible to membrane occlusion during washing of blood bank units.

- Evaluation of the AHT autotransfusion device for washing calcium-doped RBCs indicated that with a continuous flow, device sufficient, 40:1 washout could not be obtained. Calcium-doping was used in all early stage evaluation of prototypes to allow for a readily accessible indication of washout at low cost. The use of a recalculating circuit employing an autotransfusion filter has been designed and may be evaluated in Phase II.

- Evaluation of the Batch Unit Processors (BUP) demonstrated that sufficient calcium washout could be achieved. The BUP devices washed approximately a third of a unit. Of the basic configurations evaluated, the BUP I, with a single rotor gave superior results compared to the BUP II, with two rotors. The primary difference between the performance of the two configurations was a lower operating pressure with the BUP I. Based on past experience with the AHT device, a lower operating pressure typically leads to a reduction in any shear-induced hemolysis.

- Processing units of calcium-doped RBCs was performed with Whole Unit Processors (WUP) devices. The single rotor WUP I was superior to WUP I devices with surface modification with respect to transmembrane pressure and calcium washout. All WUP devices required approximately 1500 ml of wash volume to achieve greater than 40:1 calcium washout. This wash volume is essentially equivalent to the current Haemonetics 115 device.

- Washing frozen/thawed RBCs with the WUP I and WUP II/B/H devices yielded glycerol washouts of less than 1% and an average hematocrit of approximately 40% in a washing time of less than 20 min.

The TBPS is a fixed volume disposable filter with an internal chamber capable of holding a unit of red blood cells. The TBPS main filter body is comprised of a hollow, disk shaped chamber with 1.2μ Pall Loprodyne micro-pore filtering membranes forming the upper and lower surfaces of chamber. Inside the chamber is a disk rotor. The rotor is mechanically connected to a cylindrical magnet assembly that magnetically "locks" with the rotating driving magnet coupler on the Drive Unit Console. This coupling provides a closed sterile system and eliminates the need for rotating seals and the associated risks of leaks.

The spinning internal rotor creates mixing forces in the filter that aid in the cell washing process. The efficiency of the membrane is maintained by gently spinning rotors that continuously sweep past the filter membrane. The spinning rotors also impart angular momentum to the blood. Since blood flow is constrained by the casings, pressure is generated which contributes to the transmembrane pressure necessary to effect filtration. This provides a very simple method to control transmembrane pressure without complex servo-control circuits.

Phase II Proposal Goals and Summary of Progress

The Phase II Program was defined in seven experimental series. These series are summarized as follows:

1. Design of the filter
   - Disposable volume selection
   - Wash solution flowrate and rotor speed
   - Options for washing second units
2. Optimization of Operation
   - Disposable volume selection
   - Wash solution flowrate and rotor speed
   - Dilution sequence of thawed blood
   - Minimization of operator manipulation with TBPS
3. Preliminary Comparative Evaluation of TBPS vs Haemonetics 115
4. Sterility Evaluation
   - Filter sterility
   - Washed blood sterility

- Biocompatability

5. Evaluation of washing or dilution with metabolic additive solutions
6. Baboon survival study
7. Design - Construction of prototype hardware

The goal of this Phase II program was to achieve a device consisting of a disposable sterile filter washing device and console designed to wash frozen-thawed blood to the following specifications:

Glycerol washout to less than 1%
Supernatant hgb less than 150 mg/dl post washing and wash recovery of 85%
Absence of significant potassium leaks in RBC's
Hematocrit of 80% or 60% (following wash with additive solutions)
Sterile blood product
Survival of RBC's at AABB standard
Stability during storage in additive solutions for up to 5 days

The focus of the Phase II development over the past two years has been experimental series 1, 2, 3 and 7. Results presented herein chronicle the efforts to develop the TBPS by AHT. The product of this project is an integrated sterilizable disposable and an automated console which together reduce the non-machine interface requirements for deglycerolizing frozen thawed blood. Results discussed in detail later in the report indicate statistically significant improvements in the performance of the TBPS system over the 2 year period. The quality of the output blood product meets all criteria in our design specifications evaluated with the exception of the sup Hb levels averaging approximately 300 mg/dl vs our target of 150 mg/dl. In the course of development, working with our Contract Officer, Phil Gula, we did not work on Experimental Series 4, 5, or 6. As a result two specifications not evaluated were:

- Survival of RBC's at AABB standard
- Stability during storage on additive solutions for up to 5 days.

Based on previous discussions these elements are to be addressed by Dr. Meryman's Laboratory at a future date.

Experimental Methods

Standard Preparation of Blood Samples for Evaluation:

Packed Red Blood Cells: Packed RBCs are supplied by NBRL or McGuire Air Force Base. Fresh packed RBCs units are used to define optimal washing conditions with each device configuration. Blood units (300g of packed RBCs) are diluted to a hematocrit of 20-25%, the hematocrit of frozen/thawed cells following dilution prior to washing.

Thawing of Frozen Thawed RBC: The first step in the deglycerolization process is dilution with 12% sodium chloride and 0.9% sodium chloride-0.2% saline according to Valeri's method (5). 50 ml of 12% saline is added to the thawed unit while mixing on a shaker. Following this addition, the shaker is turned off and the red cells allowed to equilibrate for 2 minutes. The same procedures are followed to second (100 ml) and third (150 ml) additions of 0.9% sodium chloride-0.2% glucose to the thawed red cells. The diluted thawed cells are washed with 1.5 L of wash solution in a current revision of the TBPS disposable filter.

Washing of Thawed or Fresh Cells:

Manual Prototype: The TBPS manual prototype console allows for control of blood and wash solution flowrates, rotor speed, and the monitoring of total membrane pressure. A brief description of the procedure for washing cells with the TBPS console follows.

The filter is primed with 0.9% saline/0.2% glucose at 200 ml/min with the rotor off. For the remainder of the washing procedure the pump speed and rotor speed are maintained at values listed for each experiment in the results tables. The typical range for rotor speed is 600-1200 rpm and for flowrate between 100-150 ml/min. After priming, the blood is pumped in. When all the blood is concentrated in the filter, the saline/glucose wash is initiated for up to 2000 ml. After the wash is completed, the blood is pumped out of the filter into a satellite bag for storage.

Both fresh blood and frozen thawed cells are washed at St. Luke's Episcopal Hospital, the NBRL, and AHT Laboratory, using TBPS disposables and current revision consoles according to instructions provided by AHT and adapted from the Naval SOP. Samples collected during the thawing and washing process are analyzed by St. Luke's Episcopal Hospital (SLEH), Memorial Hospital Woodlands, or Surgimedics where appropriate.

Improvement on Manual Prototype Processing: Several changes were made during development to evaluate dependence of processing variables. A key modification was made to prime the unit with RBCs and to dilute the RBCs on line 2:1 blood: saline prior to washing.

Automated Prototype Processing: The sequence of events, shown in Table 1, includes the dilution of the thawed blood, the processing of the diluted/thawed blood and the draining of the processed blood. The firing sequence of the valves, pump, shaker, and motor determine the sequence of events.

The processing starts with the dilution of the thawed blood. The thawed blood bag is placed on the shaker. The thawed blood is diluted in the blood bag with 50 milliliters (ml) of 12% saline solution delivered at 100 milliliters per minute (ml/min) with the shaker on. The shaker is turned off automatically and the thawed blood is allowed to equilibrate for two minutes. With the shaker on, 100 ml of 0.9% saline / 0.2% glucose solution is pumped into the thawed blood bag at 100 ml/min. The shaker is turned off automatically and the thawed blood is allowed to equilibrate for two minutes. With the shaker on, 150 ml of 0.9% saline / 0.2% glucose solution is pumped into the thawed blood bag at 100 ml/min. The shaker is turned off and the thawed blood is allowed to equilibrate for two minutes.

The filter is primed with 250ml of 2:1 blood to 0.9% saline / 0.2% glucose solution delivered at 100 ml/min with the waste line open. The motor is started, and the rotor is brought up to 1400rpm. The diluted/thawed blood is pumped into the filter at a rate of 100 ml/min with a ratio of 2:1 blood to saline. Once the blood bag is empty, the saline is pumped in at 100ml/min. until there is no more saline. The filter is drained into the processed, washed blood bag and the operator is alerted. The operator seals the waste bag and discards the waste bag and disposables according to universal precautions and biohazard waste procedures. The processed blood bag is taken and used or stored for later use. Further instructions regarding the set-up and operation of the TBPS are presented in Appendix D.

Methods of Analysis:

CBC: CBC is determined on washed Frozen Thawed Red Blood Cells (FTRBC) to determine red cell number recovery, total hemoglobin, hematocrit, MCV, MCH and MCHC, and WBC and platelet counts. CBC analysis is either performed at Memorial Woodlands Hospital or St. Luke's Episcopal Hospital on a Coulter Counter or comparable analyzer.

Glycerol washout: Glycerol washout is determined by increased refractive value. It has been shown that if the residual glycerol concentration is above the desired 1%, then the measurement on a hand-held refractometer, will be in excess of 30.

Marker washout: For preliminary studies where fresh blood is used for evaluation, calcium is used as a marker for washing efficiency. The correlation between calcium washout and glycerol washout has been demonstrated. Calcium chloride is added to a unit of diluted packed RBC's prior to washing to give a concentration of 30-40 mg/dl. The washed product is then analyzed for calcium colorimetrically on a Kodak Ektachem at Memorial Hospital Woodlands..

Potassium will be measured at NBRL by flame photometry.

Calculations for Spreadsheets: Several values reported in this report's tables were derived from measured parameters. The calculations used for these are as follows:

Target hct = (blood volume in filter * pre-wash blood hct)/fixed volume of filter:
hct recovery = (post hct/Target hct/100)
Calcium washout = (sup vol out * [post Ca]/(sup. vol in * [pre Ca]) - 1 * (-100)
Post wash hgb Recovery % = (post cellular hgb/pre total hgb) X 100

Supernatant (Plasma) Hemoglobin: Supernatant hemoglobin is determined by the colorimetric method of Lijana and Williams and Standefer and Vanderjagt, using 3, 3', 5, 5' - tetramethylbenzidine as chromogen. (Sigma Procedure No. 527)

Results and Discussion

Selection of Disposable Volume

Relationship of Disposable Volume and Outlet Hematocrit

The TBPS is a fixed volume filter, therefore, the concentration of blood, i.e., the final hematocrit for washed blood will be dependent on both the volume of red cells washed and the capacity of the filter. An additional theoretical limitation is the viscosity of the blood and the effect of increased blood viscosity on shear induced from the spinning rotor in the filter.

Two filter designs have been evaluated to determine the maximum hematocrit which can be achieved in the filter disposables. The filters used were developed in Phase I, specifically the WUP I (518 pin) and BUP I (214 plain). Based on an internal volume measured to be 518 mls and 214 mls for the WUP I and BUP I, respectively, the grams of red cells placed in the device were adjusted to evaluate the maximum hematocrit of each device. Examples of such calculations are shown in Table 2a &b for each device.

The proposed uses for the BUP and WUP were to wash one or two units, respectively. During the course of the studies the approach of processing two units simultaneously in one filter was deemed as less desirable than two single units washed with the same filter in series. Results for two unit washing by each approach are discussed later in this report.

The BUP filter has a volume of 214 mls. Table 3 shows calculations of the RBC capacity of this filter at hematocrits up to 70%. The range of frozen unit volumes is significant, however, the typical range is between 200-240 grams per unit (Table 2). These unit weights convert to a cell volume of 173 to 208 per unit. Given this range it is likely that the 214 plain filter will need to be increased somewhat in volume to accommodate larger units of frozen/thawed blood. However, for the development efforts, only one additional volume was evaluated, a 320 ml filter. Results for this filter are discussed and contrasted to the 214 ml filter later in this report.

Preliminary Evaluation of 214 Plain Rotor 1.2 μm in Loprodyne (LP) Media

214 Plain 1.2 μm LP Filter Washing of Ca^{+2}-Doped RBCs:

The 214 Plain (BUPI) design holds 214 ml of volume. Preliminary tests were performed using Calcium-doped blood blank red blood cells to establish operational conditions and dependent variables. The operating conditions for each trial are listed in the results tables. Variables presented are the blood volume used, saline wash volume used, average flow rate, rotor speed, target hematocrit (theoretical hematocrit of washed blood product), a recovery calculated based on hct, calcium washout and pre and post-wash sup. hgb levels.

Evaluation of the Effect of Increasing Target Hematocrit on performance of the 214 plain filter was performed. The results are summarized in Table 4 for tests within four ranges of target hematocrit, 55%, 55-65%, 66-80% and 70-80%. Testing was performed with fresh calcium-dropped RBCs. Results in Table 4 indicate that the recovery based on hematocrit > 95% on average when the target hematocrit was between 50 and 65% (See Groups A and B). The apparent high recovery (>100%) for the Group A is an unexpected result. Such results are only seen in early experiments performed in the first quarter and there is no obvious explanation as to why these low hct tests would show such high recoveries. However, when

the target hematocrit exceeded 65%, the final hematocrit was on average appreciably less than the target (Group C and D). These results suggest that the maximum hematocrit achievable with this filter configuration is approximately 60-65%. There is an apparent relationship, between elevated pressures, increased hemolysis, and achieving post wash hematocrits less than target hematocrits. This relationship supported the need to evaluate modifications in the filter volume and rotor to address these issues.

Efficiency of marker washouts for fresh blood cells doped with $CaCl_2$ as a washout marker was determined. Results for washout for these cells washed with the 214 plain rotor are summarized in Table 5. The typical glycerol level following thawing frozen blood is approximately 40% and must be reduced to less than 1%. This equates to a washout percentage of 97.5%. For the Ca^{+2} doped RBCs washed with the 214 plain filter, 33 of 37 had washout percentages in excess of 97.5%. This result clearly indicates the TBPS in this configuration can remove $CaCl_2$ and predicts sufficient removal of glycerol frozen thawed blood under like conditions.

The Role of Transmembrane Pressure was also reviewed for all 214 plain filter tests. Table 6 lists the test results divided into groups separated as a function of maximum transmembrane pressure. Examining this data, there is a dramatic decrease in the hct recovery for tests where the pressure exceeded 300 mmHg. This decreased recovery does not correspond to an appreciable difference in post supernatant hgb levels. However, a correlation does exist with respect to the target hematocrit. The average target hematocrit for Group B is more than 10% greater than those filters which operated at pressures less than 300 mmHg. This result suggests that there is insufficient mixing by the plain rotor in the 214 plain filter to prevent partial occlusion of the filter during washing, leading to increased pressure. These results supported the transition to modified rotors to improve mixing.

214 Plain Evaluation of the Waste Flow Rate from each membrane within the TBPS was performed in two trials to determine if there was different waste flow rates through the upper and lower casing membranes. The goal being to determine if a differential in occlusion of the lower or upper membrane existed. The target hematocrit for these runs was set at 85-86 percent to exaggerate the effects of any membrane occlusion. As expected for higher target hematocrit runs the pressure was high and the final hematocrit only 52-55%.

The total flow as a function of processing time for each test is shown in Figure 1. This figure illustrates clearly that there is no divergence over time of the waste volume flow. The irregular behavior at the beginning of each run seems to be a result of displacement of air that was not completely removed during the priming. It is also interesting to note that although the area of the lower casing is less than that of the upper casing by approximately 9%, this seems to have no bearing on the waste flow rate. This is likely because the rotor sits closer to the lower membrane, leading to more effective mixing at the lower membrane surface thus compensating for the smaller area. This hypothesis supports the concept that improving the mixing at the membrane surface will improve waste flow rate and accordingly reduce pressures in the filter.

The effect of rotor speed on the performance of the 214 plain filter was evaluated by monitoring the transmembrane pressure within the filter and the waste supernatant hgb as a function of rotor speed. Two experiments were performed in which the 214 plain filter was filled with blood and washed with saline at 100 ml/min. at 1200 rpm initially followed by decreases to 1100, 1000, 900, 800, 700, 600 and back to 1200 rpm in series.

Results for the effect of rotor speed on cell washing with the 214 plain rotor filter are shown in Figure 2. These results indicate that as the rotor speed is decreased, the pressure increases, with a maximum pressure

reached at approximately 700 rpm. This result further supported the conclusion that mixing would be critical to improving filter performance by reducing occlusion of the filter membranes.

All results with the 214 plain rotor suggest that the target hematocrit and associated partial occlusion of the membrane leading to pressure increases are the dominant dependent variables with this filter type. An additional parameter evaluated is the blood and saline flow rate but did not appear to affect performance.

214 Plain 1.2 μm LP Evaluation of the Effect of Adding Blood and Saline Simultaneously

A potential optimization point was the hematocrit of the blood when entering the TBPS. The possible advantages of lowering the inlet hematocrit are reducing the viscosity for the cells as they are initially washed and increasing the overall change in hematocrit which contributes to washout. One means of decreasing the initial hematocrit was to mix the blood and saline simultaneously inline as they were pumped into the filter.

We performed two sets of trials to assess whether with the 214 Plain TBPS design this manipulation would affect the performance of the device. Results in Table 7 suggest improved post supernatant hgb levels but no other apparent improvement. The benefit of this modification was demonstrated when priming with blood was subsequently introduced (see below).

214 Plain 1.2 μm LP Preliminary Studies Washing Frozen-Thawed RBCs

Experimental Series I studies with Frozen thawed blood were completed. Nine units of frozen blood were thawed, diluted and washed with the 214 Plain TBPS filter using standard conditions of 100 ml/min flow rate and a rotor speed of 1200 rpm. Processing conditions and NBRL testing results are listed in Table 8. These results indicate that target hematocrits of up to 60% can be achieved when the pressure in the filter is kept below 110 mmHg. In four of the tests the pressure was above 200 mmHg. Three of the four of these likely showed elevated pressures due to overloading the filter with cells as evidenced by theoretical target hematocrits of greater than 80%. Steps were taken to prevent such overloading in future tests. The exception to this set was the fourth sample with high pressure which achieved its target hematocrit despite the elevated pressure. Supernatant hemoglobin levels post washing were elevated in the high pressure tests compared to the tests which operated at less than 100 mmHg.

Results suggest that all nine units had acceptable freeze/thaw recovery, validating that the procedures used are consistent with the NBRL SOP. Red cell recovery was calculated based on the amount of hemoglobin lost to the waste and on the measurement of cellular hemoglobin in pre and post wash samples. In all tests the waste hemoglobin calculation yielded higher recoveries than the pre/post analysis. A likely explanation for this discrepancy is that not all the cells were removed from the filter in some of the tests. Waste recovery results indicated that washing with the TBPS device can yield acceptable recoveries of greater than 80%. In order to improve the pre/post recovery additional steps were taken to improve the method of removing cells from the filter in future tests.

The washout of glycerol, determined by the osmolarity of the supernatant was very good for all nine units. This indicates that for the volume of blood being processed in these tests that 1000 mls of wash solution was sufficient. Two indicators of cellular damage post washing are the supernatant hgb and the intracellular potassium levels. For units washed at pressures below 150mmHg the supernatant hgb levels were well below the AABB limit of 500mg/dl. However, to improve post wash hgb levels, additional saline wash volume may have been required. Intracellular potassium was measured for three units. The measured level averaged 4.1 vs a normal of 6-7mEq/RBC. A reduction in potassium is an indication of

membrane stress. This result suggests that in the 214 Plain configuration, the TBPS potentially caused stress to the RBC membrane, likely due to shear, resulting in potassium leakage. This observation supported the apparent need to modify the TBPS rotor in order to reduce the shear. This we believed could be accomplished by improving mixing while reducing the rotor speed.

Filter Rotor Modification Evaluation

1.2 μm LP Disposable Rotor Design Options

In order to increase the final hematocrit inside the filter without observing high pressure, the membrane had to be more effectively cleared. This means the rotor had to be modified to create more turbulence (but not more shear, since shear itself leads to hemolysis).

With the 214 plain 1.2 μm LP filter, there was apparently inadequate mixing within the filter because the current rotor behaves essentially as a flat, smooth plate inside the filter. One or more simple structural modifications to the rotor should improve mixing within the filter, thus allowing greater target hematocrits while reducing internal pressure.

Previous testing results with the BUP I have suggested that the upper limit for the outlet hematocrit was 60%- 65% likely as a result of inadequate clearing of cells off the membrane which yielded over pressure in the filter. Given these results, we performed preliminary evaluation of several rotor design configurations.

In order to determine the effectiveness of different rotor configurations for mixing within the filter the vortex, pressure, and fluid velocity were observed. The fluid flow and pressure at the inlet line were evaluated for several rotor configurations: plain rotor, rotor with 4 radial bars, rotor with 4 straight bars at an angle to the radius, and rotor with 4 ridges with kinks in each bar to approximate a smooth windmill design. It was found that the rotors with straight ridges caused the greatest fluid velocity for a given rotor speed. However, the curved shape of the bars provided centripetal force that served to decrease the centrifugal pressure on the outer rim of the filter and increase the pressure in the low pressure area in the center of the filter, thus equalizing the pressure in the filter. Further testing was performed with a 12 ridge windmill configuration which demonstrated that this design appeared to optimize mixing.

During the evaluation of new rotors, it was determined that the bond strength of the membrane to the upper and lower casing plastic was insufficient to handle the forces and pressures generated. To solve this we evaluated several support media and selected one which provided efficient bond strength.

Based on these preliminary results several rotor configurations were evaluated for washing blood this quarter. Each different rotor design was tested with the 214 plain filter as a control. Initial studies with modified rotors were performed with calcium-doped fresh RBCs.

The initial modified rotor studies evaluated the effect of three modified rotors on performance of the TBPS filter. The filters evaluated included:

a. 214 plain - the original flat rotor
b. 214 3R - a flat rotor with three vertical fins positioned on each side of the rotor, perpendicular to the rotor edge at 0, 120, and 240 degrees
c. 214 3R 2H - a 214 3R rotor with two holes drilled in the rotor to allow for mixing between each side of the rotor

d. 214 12R 6H - a flat rotor with 12 ridges on each side of the rotor, arranged in a curved pattern to improve mixing and reduce shear, and with 6 holes in the rotor

Review of the results (listed in Table 9) and comparison of the results obtained with each type of filter rotor design indicate that modifications to the rotor could improve performance for RBC washing. All tests we performed with high target hematocrits to maximize any effects from membrane occlusion. 214 plain filter results for similar hematocrits are listed for comparison. Results with the 214 3R rotor in the 214 filter compared to the 214 plain filter suggested that the addition of three ridges on each side of the rotor did not improve the target hematocrit or operating pressure during washing of higher hematocrit samples. However, results for the 214 3R 2H disposable, in the one filter evaluated, suggested that the presence of the holes increased the output hematocrit and decreased the operating transmembrane pressure. This combined effect of ridges on the rotor surface and the holes in the rotor lead to the design of the lead prototype filter, the 214 12R 6H, a 214 ml filter with a rotor with 12 ridges on each side of the rotor and 6 holes drilled in the rotor.

The 214 12R 6H rotor 1.2 μm LP was designed to maximize mixing and allow for lower rotor speeds and shear. As seen in preliminary testing, the 214 12R 6H disposable showed a further increase in the output hematocrit and operating pressure at an elevated target hematocrit. While the target hematocrit was not achieved on average (Table 9), note that the operating pressure was lower compared to other modified rotors with fewer ridges or holes.

Previously, with the 214 plain rotor disposable, increasing the flowrate frequently lead to an increase in the pressure within the filter and an apparent increase in hemolysis. In two preliminary experiments, 102 and 104, (see Table 10) we varied the flowrate from 100 up to 200 ml/min during the washing of calcium-doped RBCs with the 214 12R 6H and did not observe an effect on the transmembrane pressure. This suggested that the variables which affected the original 214 plain rotor disposable may not be as dependent with the modified rotor disposable.

Given this we designed an experiment in which we evaluated the effect of rotor speed vs. the transmembrane pressure and waste plasma hemoglobin concentration at two set flow rates, 100 and 150 ml/min for washing fresh RBCs with the 214 12R 6H rotor disposable. We initially set a rotor speed of 900rpm for all the modified rotor disposables. The protocol for this experiment was to pump RBCs sufficient for a 60% hematocrit into the disposable, begin washing with the rotor at 900rpm, after equilibration the rotor speed was changed to 1000, 1100, 1200, 900, 800, 700, 600, 500 and 900 rpm for 3 minutes at each rotor speed in series. The transmembrane pressure was monitored each minute and waste supernatant samples collected immediately before changes in rotor speed following equilibration at each rotor speed.

Figure 3 represents results for washing RBCs comparing the effect of rotor speed vs. the transmembrane pressure and waste plasma hemoglobin with fixed flowrates of 100 and 150 ml/min, respectively. The lines presented represent the average of four experiments performed for each flowrate according to the above described protocol. It is impressive to note the similarity in the pressure vs rotor speed curves for each test, at a flowrate of 100 ml/min. (Figure 4). Reviewing Figure 3 several observations can be made:

- The general shape of the pressure and waste hgb as a function of rotor speed is similar and distinctly different from that observed with the 214 plain rotor filter (Figure 2).
- The pressure increased from 900-1200rpm, decreased back to starting pressure upon return to 900rpm, dropped to a low value of 600-800 rpm, and increased significantly as the rotor speed was decreased to 300-500 rpm. It should be noted that once high pressures were achieved from low rotor speed, upon

increasing the rotor speed to 900rpm the pressure did not return to the baseline level. This is probably due to cellular occlusion of the membrane from the inadequate mixing at low rotor speeds.

- The waste hgb levels decreased dramatically independent of flowrate when the rotor speed was decreased from 900 to 800rpm, with a minimum level of hemolysis at 500rpm.
- Comparing the pressure and waste hgb curves there is an apparent tradeoff between obtaining sufficient mixing to minimize pressure and limiting hemolysis from shear.
- This rotor design allows for washing at flowrates up to 150 ml/min at reduced rotor speeds, transmembrane pressure and hemolysis.
- The waste hgb levels were higher for RBCs washed with a 100 ml/min flowrate vs the 150 ml/min flowrate. This is likely an artifact from the increased wash volume at the higher flowrate resulting in a lower residual hgb in the filter at the point of equilibration of pressure and start of the experiment. In future experiments this artifact will be eliminated by washing cells until the hgb level normalizes at the initial rotor speed, this will allow for more accurate measurement of changes in waste hgb as a function of rotor speed.

These results indicate that the rotor modifications in the 214 12R 6H rotor filter did improve performance compared to the original 214 plain rotor filter using the 1.2 μm LP filter media.

These experiments were repeated with the rotor speed adjusted following an equilibrium period at the beginning of washing to obtain a baseline waste hgb level. Results for this experiment are shown in Figure 5. The results are very similar with or without the equilibrium period suggesting that the dramatic decrease in supernatant hgb at between 900-800 rpm was real and not an artifact due to lack of equilibrium (Compare Figures 3 and 5).

214 12R 6H 1.2 μm LP Disposable Results

Evaluation of the 214 12R 6H filter performance was made relative to operational parameters. Results of these parameters are discussed below.

The effect of target hematocrit on performance is listed in Table 11. These results are similar to the 214 plain rotor in that as the target hematocrit increased the hematocrit recovery decreased. This result does not clearly correlate to the other operational parameters, such as flowrate and rotor speed. However, for all samples with target hematocrits greater than 70%, the transmembrane pressure was increased relative to test with hematocrits less than 70%. The rise in pressure, with higher hematocrits did not increase or affect post-wash supernatant hgb levels on average.

In addition to the decreased hematocrit, during testing we observed larger than expected post wash volumes under certain conditions. It was determined that a 20-30 ml increase in the volume of the filter could be observed as a function of transmembrane pressure, resulting in variable actual vs. target hematocrits. To reduce this volume change we evaluated using a stainless steel clam shell to encase the filter. A summary of the results with fresh blood testing with and without the clam shell are listed in Table 12.

There was a dramatic increase in the transmembrane pressure when the clam shell was used, set B compared to set A, with each run at a 100 ml/min flowrate. These results suggest that the addition of the clam shell does prevent swelling of the filter and leads to increased pressure. The target hematocrits vs. actual were equivalent for the 100 ml/min flowrate samples on average (Sets A & B independent of the clam shell). In contrast, post-wash hemoglobin increased three fold with cells washed at 100 ml/min with the clam shell vs. without (Sets A vs. B).

The dependence of increased pressure on performance is not only limited to tests performed with the clam shell (Table 13). These results which include samples processed with and without the clam shell further support a relationship between higher (>90%) target hematocrits, elevation in post-wash supernatant hgb levels, and increased transmembrane pressure. Two of many possible interpretations is that:
a. Hemolysis leads to occlusion and increases the pressure which increases filter volume thereby decreasing actual hematocrits relative to target or
b. Pressure from blood occluding the filter at higher target hematocrit causes hemolysis and the higher pressure leads to increased filter volume thereby decreasing actual hematocrits relative to target hematocrits. We believe the former to be the most likely explanation of our results. The benefits of controlling filter volume at the expense of increased pressures will be investigated further.

Pressure within the filter is also a function of flowrate, the higher the flowrate of solution into the filter, the higher the pressure. We have determined flowrates of 100 ml/ml show lower supernatant hgb levels (Table 14).

The efficiency of the 214 12R 6H with respect to marker washout was also assessed. Table 15 tests a summary of calcium washout results for calcium-doped fresh red blood cells washed with the 214 12R 6H filter. The washout in all tests was greater than 99%, a significant improvement over the acceptable washout for the 214 plain rotor (Table 5). This result further confirms the benefits of rotor modification on performance with the 1.2 μm LP disposable.

214 Modified Rotors Part II

While the 12R 6H rotor has shown improved results vs. the plain rotor we continued our evaluation of alternative rotors. The rotors tested in the 214 ml filter disposable included:

A) 214 plain - the original flat rotor
B) 214 plain 6H- the original flat rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor
C) 214 12R - a flat rotor with 12 tubing fins positioned on each side of the rotor
D) 214 6B - a flat rotor with 6 rigid plastic blades positioned on each side of the rotor
E) 214 6B 6H - a flat rotor with 6 rigid plastic blades on each side of the rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor
F) 214 12B 6H - a flat rotor with 12 rigid plastic blades on each side of the rotor with six holes drilled in the rotor to allow for mixing between each side of the rotor

Each of these filters were compared to 214 12R 6H rotors in using matched pooled blood units each washed with different filters with various rotor configurations. Table 16 lists results for 6 sets of these data, sets A-F. Observations which can be made from these results include:

- Flat rotors (Sets A, B) yield lower actual vs. target recoveries than rotors with ridges or blades (sets C-F).
- Filters with flat rotors (Sets A, B) lead to lower post wash supernatant hgb levels vs. filters with rotors with ridges or blades (sets C- F).
- Filters constructed with blades vs. ridges (Sets E and F) caused increased hemolysis as evidenced by elevated post-wash hgb levels. These results indicate that a smoother ridge vs. a blade configuration will be required to increase the mixing ability of the rotor.

All experiments were performed with the clam shell configuration, therefore pressure was elevated in each configuration.

Another component of the rotor type evaluation was to monitor the waste supernatant during the washing for hgb levels. Figure 6 shows a plot of supernatant hgb, monitored at the waste line vs. the washing time. The results shown here indicate several interesting phenomenon. First there is a sharp peak in the supernatant hgb with all rotor types tested except the 6B rotor which shows a broad peak. This elevation in supernatant hgb may be due to hemolysis of the initial cells pumped into the filter and diluted into the saline used for priming the filter. Priming with the dilute blood is to be evaluated to determine if this supernatant hgb peak can be reduced. Second, the post-wash supernatant samples have significantly higher hgb levels for two of the configurations compared to the others and much higher than the hgb levels of the final waste line sample. This suggests that the membrane may be somehow selectively retaining hgb and passing saline during washing. This is supported also by the relative lack of difficulty removing calcium or glycerol compared to hgb with this device.

Based on these results we evaluated the 214 12R and the 214 6R 6H rotor configurations in addition to the 214 12R 6H filter for washing frozen-thawed cells.

214 12R 6H 1.2 μm LP Disposable Frozen-Thawed Blood Results

Twenty-two frozen-thawed units were processed with 214 ml filters with several rotor designs. Table 17 is a compilation of all tests performed and the data obtained from the NBRL laboratory testing for the 214 ml filters. For all these filters independent of rotor type and operation conditions the average recoveries were 71.9 and 66.9%, respectively by waste hgb and pre/post hgb recovery calculations. The post-wash supernatant hgb for all samples averaged 694mg/dl. While these results are not sufficient to meet the desired objectives of the program, we believe with continued development, we will be able to meet the objectives. The following tables evaluate the results based on the effect of operating conditions; wash volume, estimated by the total waste volume; transmembrane pressure and rotor design configuration.

Operating Parameters: Table 18 compares the effect of flowrate on the performance of the device in washing frozen-thawed cells. These results, on average suggest the following:

a. Increasing the flowrate increases hemolysis as indicated by decreased recovery and relatively elevated post-wash supernatant hgb. This is consistent with the fresh blood results discussed earlier;

b. Pressures are elevated with lower flowrates. However, all samples tested after 3/4/96 were washed in filters encased in the clam shell. This likely accounts for the increase in the pressure as was seen with the fresh cell results;

c. Wash flowrate had no effect on intracellular potassium levels on average.

Wash Volume: The wash volume is related to the waste volume. Table 19 compares results for washing frozen-thawed cells with the 214 12R 6H filter based on three groupings of waste volume; less than 2000 ml, between 2000-2500 ml, and greater than 2500 ml. Reviewing the results, only intracellular potassium appears to be potentially decreased as the wash volume was increased. All other parameters appear to be independent of wash volume based on this data.

Transmembrane Pressure: Elevation in the pressure has been discussed previously as a potential cause of hemolysis and decreased recovery based on hematocrit with fresh cells. Table 20 compares results for washing frozen-thawed cells with the 214 12R 6H filter grouped in sets according to the maximum pressure achieved during the wash process. The only parameter which may be dependent on the pressure is the recovery. Somewhat unexpectedly, the recovery appeared to increase with filter transmembrane pressure elevation, with the highest average recovery observed for Sets C and D with maximum pressures in excess of 400mmHg.

Rotor Design: Two additional rotor configurations have been evaluated compared to the 214 12R 6H rotor using pooled frozen-thawed blood. Table 21 lists three sets of results for pooled tests. The filters evaluated with frozen blood were the 214 12R and the 214 6R 6H rotor configurations. Comparison of the results in each set suggests that reducing the number of ridges may improve the recovery and decrease hemolysis as evidenced by post wash supernatant hgb (sets B and C).

Optimization of the Washing Process and Disposable Configuration

Two visits to Dr. Valeri's Laboratory at the NBRL in Boston were made during March and May of 1996. As a result of these visits several modifications to the washing process were made including:

- modifying the waste port
- priming the filter with diluted blood
- diluting in-line at a ratio of 2 parts diluted blood to 1 part wash solution
- introducing blood and wash solution through the top of the filter.

The rational for each of these modifications is as follows:

- The waste port was moved closer to the center of the filter in order to evacuate air more effectively, which increased effective membrane surface area for the top membrane.

- Introducing thawed, diluted blood into a filter primed with only wash solution induced osmotic shock, causing hemolysis. Therefore, in the modified configuration the filter was primed with diluted blood.

- In order to further reduce osmotic shock, the blood was diluted with wash solution in-line at a ratio of 2:1 (blood:wash solution).

- In order to reduce shear stress on the blood as it enters the filter, the blood and wash solution were introduced at the top of the filter rather than the side.

Results for preliminary tests performed with the modified configuration were obtained from two different pools of thawed frozen blood (Table 21). The recovery using waste hemoglobin (hgb) and recovery using pre/post hgb measure the percentage of cells that survive deglycerolization. The post supernatant (sup) hgb., a measure of the concentration of free plasma hgb retained in the washed unit, is presented in Table 21 as a performance parameter. The hgb released is an indicator of the amount of hemolysis produced in the process. The formula for hgb released is:

hgb released = (post total sup. hgb) + (waste total hgb) - (pre total sup. hgb)

Table 22 illustrates that the modified configuration performs better than the unmodified. For the modified configuration recoveries were higher, post sup hgb levels were lower, and hemolysis levels as measured by hgb released were lower. Table 23 further demonstrates the effective increase in performance that the modified configuration provides. The data summarized compares results with the unmodified 12R6H, modified 12R6H and modified 3R6H (3 ridge, 6 hole rotor) rotor configurations obtained with n thawed frozen blood tests.

The recoveries, post sup hgb levels, and hgb released levels were improved not only in the modified configuration but also in the modified configuration with the 3R 6H rotor. An analysis of variance (ANOVA) was performed at a 95% confidence limit on the data and indicated a statistical significance difference between groups in all categories (Appendix A-1). The modified process and disposable configuration decreased the osmotic shock on the cells, decreased the shear on the cells, and increased the effective membrane surface area. Therefore, the modified process and disposable configuration was incorporated as the standard procedure.

Rotor Design with Modified Process using 1.2μm LP Disposable

Given the improvement with the modifications to the washing process suggested by Dr. Valeri, we reevaluated the effect of rotor type on performance.

Three rotors were tested:

- 12 ridge, 6 hole (12R 6H)
- 6 ridge, 6 hole (6R 6H)
- 3 ridge, 6 hole (3R 6H)

As is observed in Table 23, the 3R6H rotor appeared to yield improved results compared to the 6R6H rotor. The rotors differ in their effective mixing ability and production of hemolysis from fluid shear. The 12R 6H rotor has the greatest mixing ability coupled with the greatest production of hemolysis from shear. The 3R 6H rotor creates less hemolysis from shear coupled with the least effective mixing ability. The 6R 6H is in between the 12 R 6H and 3R 6H in both effective missing ability and production of hemolysis from shear.

A comparative test was performed with six different pools of thawed frozen blood to compare the three rotors. The results from these tests were averaged over the number of tests, N, and are presented in Table 24.

The performance of the 12R 6H was comparably worse than both the 6R 6H and 3R 6H in all the categories of recovery, post sup hgb, and hgb released. Table 24 suggested that increased hemolysis from shear in the 12R 6H rotor overrides the increased effective mixing ability. An ANOVA was performed at a 95% confidence limit on the data providing statistical significance that the 6R 6H and 3R 6H performed better than the 12R 6H in all categories; however, there was no statistically significant difference between the performance of the 3R 6H and 6R 6H rotors (Appendix A-2). However, prototyping times on the 3R 6H are shorter than prototyping times on the 6R 6H, making the 3R 6H the most cost effective choice. Therefore, the 3R 6H rotor was selected as the standard configuration.

Filter Media Optimization

Early in Phase I studies, several filter media types were evaluated. Based on that evaluation, the 1.2 μm Pall Loprodyne (LP) membrane was selected. The media used for all tests with thawed frozen blood in previous quarters were either 1.2 μm or 3.0 μm Loprodyne.

We also evaluated the benefit of using support media in conjunction with the filtration media. Recognizing the need to improve performance with respect to sup hgb and concentration, we reevaluated filtration media. Three types of media were investigated: depth tortured path, microporous, and screen media (Figure 7). Depth tortured path media are high porosity membranes with a thickness greater than 100 microns (μm) which separate particles by torturous paths with variable pore size and shape. The 1.2 and 3.0 μm rating for the LP membranes is based on passage of specified microorganisms of defined size. Microporous media are characterized by a thickness of 10 to 25 μm, straight paths for particulate, defined pore size and shape, and low porosity. Screen media are characterized by a thickness of 25 to 150 μm, straight paths for particulate, defined pore size and shape, and high porosity. Figure 7 illustrates the difference between the types of media. Microporous and screen media have advantages over tortured path media because of minimal depth (thin = fast diffusion), straight paths, and defined pore size. Screen and

depth tortured path media have the advantage over microporous media because of their high porosity. Appendix B lists all media investigated classified by media type.

Results for the evaluation of alternative media with the 3R6H rotor disposable consisted of the following comparative studies:

- 1.2μm vs 3.0μm Loprodyne media
- 1.2μm LP with full vs partial T-106 support media
- 3.0μm LP vs Pall Biodyne media
- 1.2μm LP vs Pall Biodyne media
- 3.0μm LP vs 1μ Whatman Cyclopore polyester CPC - both with full T-106
- Pall 3.0μm LP vs 1.0μm Whatman Cyclopore PET, both with full T-166.
- Pall 310μm LP vs 1.0μm lWhatman Cyclopore PET unsupported (no T-106)

1.2μm vs 3.0μm LP media

The media differ in their ability to effectively allow stroma and free sup hgb to pass from the filter to the waste. The 3.0μm media, by virtue of its larger pore size, is able to pass troma and free sup hgb more easily than 1.2μm. Tests were performed on both 1.2 and 3.0μm media with 4 different pools of thawed frozen blood. The results from these tests were averaged over the number of tests, n, and are presented in Table 25. The maximum pressure is the greatest internal pressure of the filter during processing and is recorded in millimeters of mercury (mm Hg).

T-tests were performed on all the performance parameters at a 95% confidence limit between the 1.2 and 3.0 μm media (Appendix A-3). The maximum pressure and post sup hgb were the only performance parameters that were found statistically different between the 1.2 and 3.0μm media. The lower post sup hgb values of the 3.0μm media support the assertion that 3.0μm media allows stroma and free sup hgb to pass from the filter to the waste more easily than the 1.2μm. In addition, stroma was observed in the waste of 3.0μm tests but not in that of 1.2μm tests. The higher internal filter pressure in the 3.0μm tests prompted further testing of the effects of pressure on hemolysis.

Support Media: Full versus Partial T-106

Pall T-106 has been used as a support media to strengthen bonding of filtration media. T-106 is a spun nylon 40 μm porous support. Most previous studies have used partial T-106; however, the process of preparing partial T-106 was cumbersome. The use of full T-106 to support the filtration media would significantly reduce prototype filter assembly time. Full and partial T-106 are illustrated in Figure 8.

Raw performance data were analyzed using paired sample t-tests with a significance level of 5%. Selected results presented in Table 26 indicate that thawed blood washed with filters using partial and full T-106 with 3.0 μm LP are equivalent. Results are summarized in Appendix C, Table 1. No statistically significant difference was found in the performance of the two. The filter media used in these studies was 3.0 μm LP. The lack of statistically significant differences between the performance of the full and partial T-106 led us to proceed in future studies with full T-106 to allow for ease of filter assembly for media requiring support.

Alternate Media: Pall Loprodyne versus Pall Biodyne A

Pall Loprodyne and Biodyne A (BA) membranes are nylon depth tortured path media prepared by the same process. Loprodyne has a surface treatment, while Biodyne A does not.

3.0 μm LP versus 3.0 μm BA Selected results presented in Table 7 compare 3.0 μm LP to 3.0 μm BA media (summary data is found in Appendix C, Table 2). Analysis of these results indicated statistically significant differences between the performance of LP and BA media. Biodyne A statistically outperforms Loprodyne with respect to post hct and maximum pressure, but no statistically significant difference was found between the performance of LP and BA with respect to post sup hgb. The lower pressures correspond to higher hcts because of reduced expansion of BA filters.
1.2 μm LP versus 1.2 μm BA Results comparing the 1.2 μm LP and BA media are shown in Table 28 (summary data is found in Appendix C, Table 3). Reviewing these results, no statistically significant differences were observed.

Based on these studies, we chose not to pursue the 3.0 μm and 1.2 μm pore Biodyne media in preference to the Loprodyne media. Evaluation of additional media was continued.

Alternate Media: Pall Loprodyne versus Whatman Cyclopore

Whatman Cyclopore membranes are microporous media prepared by an acid etching and ion bombardment process that creates right circular cylindrical paths. We evaluated Cyclopore to determine the impact of a microporous media on performance. We compared 3.0 μm LP to 1.0 μm Cyclopore polyester (PET) and polycarbonate (PC), both supported with full T-106. We also compared 3.0 μm LP to unsupported 1.0 μm Cyclopore PET. Attempts were also made to compare 2.0 and 3.0 μm Cyclopore PET and PC to LP. However, the 2.0 and 3.0 μm Cyclopore pore sizes proved to be too large and allowed RBCs to pass, whereas the 1.0 μm pore size retained RBCs.

Pall 3.0 μm LP vs. Whatman 1.0 μm Cyclopore PC, Both with full T-106 support Five pools of thawed frozen blood were processed to compare the performance of 3.0 μm LP and supported 1.0 μm Cyclopore PC. Results are shown in Table 29 and Appendix C, Table 4. No statistically significant difference was observed between the performance of LP and supported Cyclopore PC.

Pall 3.0 μm LP versus Whatman 1.0 μm Cyclopore PET, Both with Full T-106 Support Seven pools of blood were processed to compare the performance of 3.0 μm LP and supported 1.0 μm Cyclopore PET. Results are shown in Table 30 and a complete summary is found in Appendix C, Table 5. There was no statistically significant difference found between the performance of LP and supported Cyclopore PET with respect to post sup hgb. Higher hcts correspond to smaller filter volumes which were determined by lower pressures (see section *Internal Filter Pressure Effect on Volume and Hematocrit*). Results with supported 1.0 μm Cyclopore PET were encouraging but were not sufficient to warrant a change in media from Pall 3.0 μm LP.

Pall 3.0 μm LP versus Unsupported Whatman 1.0 μm Cyclopore PET During bonding studies it was observed that T-106 is not required as a support for 1.0 μm Cyclopore PET. We evaluated performance of the supported 3.0 μm Pall LP and 1.0 μm Cyclopore PET without T-106 support using four pools. Results are shown in Table 31 and Appendix C, Table 6. The unsupported 1.0 μm Cyclopore PET statistically outperforms the 3.0 μm Pall LP with respect to post sup hgb, hct, pressure, recovery using waste hgb, hgb

released, and hgb retained. The hgb retained is the mass of hemoglobin in the processed unit, calculated from the volume of plasma and concentration of hemoglobin in the plasma.

The difference between mean pressures of 3.0 μm LP in Table 27 (388 mmHg) and 3.0 μm LP in Table 31 (901 mmHg) is due to the use of different sized units. Reduced size units (154 to 180 mL RBCs) were used in 3.0 μm LP versus 3.0 μm BA (Table 27) to highlight the difference in performances between depth media. Full size units (178 to 191 mL RBCs) were used in 3.0 μm LP versus unsupported 1.0 μm PET (Table 31) runs to test the capabilities of the new media. Excessive pressures were not seen with the unsupported PET media (mean of 289 mmHg), in contrast to the 3.0 μm LP.

Higher hcts correspond to smaller filter volumes which were determined by lower pressures. The smoother surface and defined pore structure of the PET membrane may contribute to reduced hemolysis as evidenced by the reduced hgb released values. Based on these results we are switching to unsupported 1.0 μm Cyclopore PET as the standard media.

Optimization of Design & Process Parameters for 1.0 μm PET media

Several additional studies were undertaken to optimize performance of the 1.0 um PET media in an effort to reduce supernatant hemoglobin levels post processing:

- Rotor selection and rotor speed
- Sealing the magnet well
- Alternative thawing - dilution procedures
  - use of 12.0/0.9% to 8.5% / 1.6% saline dilution schemes
  - warming saline
- Improvements in mixing and reduction in blood hold-up spaces

Rotor Selection and Rotor Speed

AHT re-evaluated the plain flat rotor for its effect on performance. Appendix C, Table 7 lists all results for testing with the plain rotor at speeds of 900-1800 rpm. Table 32 summarizes the effects of 1200-1800 rpm on post hematocrit, post supernatant hemoglobin, waste hemoglobin recovery percentage and pre/post hemoglobin recovery percentage. Results indicate the recovery decreased at 1800 rpm compared to 1200, 1400, or 1600rpm. All recovery and supernatant hemoglobin levels were equivalent between 1200 and 1600 rpm. The only statistically significant difference in performance was a lower hematocrit at 1200 rpm vs. 1400 rpm. Based on these results we selected 1400 rpm as the mid-point of the 1200-1600 rpm range for our standard rotor speed for future studies.

In order to verify the decision to move to the plain rotor from the 3 ridge (3R) rotor we also evaluated the 3R rotor at 800 & 1000rpm. Complete results are compiled in Appendix C, Table 8. Table 33 lists results for post hematocrit, post supernatant hemoglobin, and waste hemoglobin recovery and pre/post hemoglobin recovery. With the exception of post-hematocrit, all other parameters appear equivalent for 900 and 1000 rpm with the 3R rotor. Comparing these results to the plain rotor at 1400 rpm a dramatic (50%) decrease in post supernatant hemoglobin was evident (616 mg/dl for 1000 rpm 3R rotor vs. 342 mg/dl for 1400 rpm plain rotor)

Magnet Seal

A potential method for improving performance and recovery of blood post washing is to place a seal at the top of the magnet well to prevent blood from filling the narrow gap between the magnet and the well wall. Two types of seals were evaluated, a 0.005 and a 0.010 thickness. Four disposables with each of these seals were assembled and performance was evaluated versus the standard disposable using 4-three unit pools. Compiled results are listed in Appendix C, Table 9. Results for these tests showed the seals we used did not improve performance with respect to recoveries, post-supernatant hemoglobin, and post hematocrit (Table 34).

Based on these results alternative materials and methods for sealing the magnet will need to be investigated in Phase III.

Dr. Meryman's Review & Evaluation

AHT was visited by Dr. Meryman in November 1996. Several potential areas for improvement were identified for evaluation:

1) 8.5 %/0.9% dilution sequence
2) effects of warming saline
3) improved mixing of blood and saline in-line prior to entering the disposable filter
4) seal all areas where unwashed blood could settle

Diluent Comparison, 12.0/0.9% vs. 8.5/1.6%

Based on published results Dr. Meryman has concluded the two dilution sequences yield similar results. We evaluated both diluents (12.0/0.9% vs. 8.5/1.6% saline) in several paired two unit pool studies. Table 35 lists average results for post supernatant hemoglobin, waste hemoglobin, and waste hemoglobin recovery. Results with the TBPS system showed that the 12.0/0.9% diluent was statistically better to the 8.5/1.6% diluent for each of these parameters. Additional results are listed in Appendix C, Table 10.

This difference between the diluent methods with the TBPS was not predicted based on previous results published by Meryman. The choice of diluent will be addressed further by Dr. Meryman during planned comparative studies to be performed following completion of Phase II.

Warm vs Room Temperature Saline

After thawing the glycerolized blood is at 37°C. Elevating the temperature of the saline in addition to the blood can have several effects including reducing the viscosity of the solution and increasing glycerol transfer across the membrane. Results from 4 two pooled unit studies in Appendix C, Table 11 did not show a statistically significant advantage using warmed saline in any parameter evaluated. In these studies saline was warmed to 37°C with the blood prior to processing. However, the results did suggest a non-statistically significant improvement in all of the following parameters (Table 36). Based on these results the use of pre-warmed saline for washing was added as a component of our standard protocol.

In line mixing and sealing to prevent settling of unwashed blood

Two additional potential improvements were evaluated in two studies using three unit pools. Each study consisted of one unit as a control (with standard in line mixing, unwarmed saline wash solution, and the standard disposable configuration) and two additional units processed with warm saline wash solution under the following conditions.

Study 1:
1. Modified mixing with a reverse drip chamber planed in-line to mix blood and saline and standard disposable configuration.
2. Modified mixing (same as 1) with sealing void volumes in disposable.

Study 2:
1. Modification of disposable to seal void volume without modified mixing in-line.
2. Same as 2 above.

Table 6 highlights several parameters and notes the decrease in post supernatant hemoglobin for each study when the combination of process changes were utilized. Appendix C, Table 12 lists additional results for these studies. Based on these results, we elected to make this combination of processing changes standard for further studies.

Single Unit Washing with Manual Console

Given the apparent improvement using the combination of processing modifications evaluated in Tables 36 and 37, we decided to evaluate where we were with respect to processing single units without pooling. Appendix C, Table 13 lists results for seven units processed using the parameters identical to the mix and clamp combination in Table 37. These results showed dramatic improvement in performance with respect to post supernatant hemoglobin (average 219 mg/dl) while maintaining recoveries in the 85% range (Table 38) compared to previously reported results.

The individual units selected covered a wide range of RBC volume (128-205 ml) and presupernatant hemoglobin levels (919-7289 mg/dl.) These results may suggest:

- The larger units had greater post-thaw supernatant levels. This may be due to insufficient glycerol added at the time of freezing relative to the blood volume.
- The volume of blood in the disposable directly influenced the maximum pressure.
- Elevated post-thaw supernatant hemoglobin levels did not impact final post-wash supernatant hemoglobin levels.
- Figure 9 plots the relationship between maximum pressure and post-wash recovery. The waste hemoglobin recovery showed a decrease as a function or pressure. However, when the waste hemoglobin was corrected for post-thaw recovery this effect is nullified.
- The quality of post-thaw blood dramaticaly affected the recovery. When the recovery was corrected for the supernatant hemoglobin post thaw, the recoveries ranged from 89-95% indicating the device can yield consistent recoveries of >89%.

Single Unit Washing With Automated Console

Having finalized the disposable configuration and operation with the manual console, we completed the assembly of the automated console and repeated single unit testing to confirm the results on Table 38 above. Results in Table 39 are divided in two parts. Part A and B list results for individual units with pre-supernatants of ≤ 5000 and >500 mg/dl, respectively. Results for units washed with the automated console are equivalent to results with the manual console (compare Table 38 vs. 39a). Reviewing the results, it is apparent that excessive hemolysis (>5000mg/dl) leads to poorer performance. The poorer results are for units with elevated pre-supernatant hgb and are likely due primarily to the relatively poor quality of the thawed blood product. While hgb washout did not break 200mg/dl on average, these results do indicate that the TBPS can wash frozen/thawed units with low and high supernatant hgb and yield excellent washout on a percentage basis. These results also indicate the importance of quality freezing and thawing with respect to their affect on pre-wash supernatant hgb values and recovery post-thaw.

Based on these results it appeared the TBPS system should be able to process a wide range of unit sizes and yield an acceptable blood product.

Miscellaneous Studies

Increased Filter Volume

The results with the 214 12R 6H filter lead us to begin preliminary evaluation of washing large unit volumes of blood with a 320 ml filter configured with this rotor. The goal was to determine if increasing the filter size would affect performance with a 12R6H filter. The filter's internal volume was increased by adding to the height of the center ring. Increasing the ring height also required an increase in the height of the rotor in order to keep it in the center of the casings.

We evaluated the effect of rotor speed vs. the transmembrane pressure for the 320 12R 6H rotor filter at both 100 and 150 ml/min flowrates. Results for these experiments are shown in Figure 10. These results are encouraging, since the pressure vs rotor speed with the 320 ml filter is comparable to the 214 ml filter. There appears to be a small increase in the operating pressure at a 150 ml/min flowrate with the increased filter size. These results suggest that increasing the flowrate above 100 ml/min for routine washing of a full unit may be possible.

The 320 12R 6H rotor has also been evaluated for routine washing of calcium-doped RBCs. Results of tests are listed in Table 40. In 4 of 5 of these experiments the target hematocrit of 60% was met. This hematocrit is consistent with those achieved for 214 filters. The pressure was low in each filter. Post-wash supernatant hgb results were mixed. These results strongly suggest that with modified rotors, such as the 12R6H, increasing the final production filter volume to accommodate the "average and range" of frozen blood units should be possible.

Two Unit Washing

One of the issues we have addressed is how to wash a second unit with the same filter. Two approaches have been examined.

a. Washing two units simultaneously in one filter, yielding a super unit.
b. Back to back washing in series in a filter which holds one unit.

Simultaneous Washing The original intent was for the 518 ml plain filter (WUP) to be used for washing a single full unit. With the change in final hematocrit specification from 40% to 60-70% from the Phase I to Phase II of this program, the 518 plain filter became obsolete for single unit washing. However, it did allow for evaluation of washing two units simultaneously in one filter.

Results from four tests with the 518 plain rotor suggest that the filter as designed did not give adequate performance with a maximum actual hematocrit achieved of less than 55% (Table 41). This approach has not been pursued further due to the results and a preference for washing units in series compared together.

Washing in Series in the same filter. To wash two units back to back in the same filter. We have evaluated washing two units with the 214 plain disposable and the 214 12R 6H disposable (Table 42). Results with the 214 plain filter showed decreased recovery and increased transmembrane pressure for the second units washed. We investigated whether the improved mixing in the 214 12R 6H filter would allow for such washing. Experiments 114/115 indicated that the improved mixing did not significantly improve the performance of washing a second unit due to increased pressure and decreased post-wash hematocrit. The likely cause for this reduction in performance was debris occluding the membrane, which was not removed in flushing the filter between units.
In an attempt to address this a backflush procedure was developed for washing the filter following washing the first unit. This was performed by gravity feeding 1l of saline through the waste line to backflush the membrane with the rotor spinning at 300rpm. The flushed debris was collected in a satellite bag for disposal. Experiment 128/129 was performed in this manner using the 214 12R 6H rotor and show that for the first time comparable results are obtained for the first and second units washed. Given this result we repeated the experiment with the 320 12R 6H rotor filter. These results for experiment 130/131 were similar, with little increase in pressure and only a slight decrease in the post hematocrit, relative to the first unit.

Following the completion of the filter disposable development we reevaluated the ability to wash two units in series. While recognizing we had previously demonstrated that a back flush procedure could be performed and clear the membrane to allow comparable washing of both units, we first evaluated testing without a rinse procedure between units or/the revised filter. The disposable configuration used for these studies was the 1μm Cyclopore PET media plain rotor filter disposable with side feeding. Results in Table 43 a&b compare the performance for each unit. Comparing first and second unit results there is no appreciable difference between the performance of the disposable for each unit. These results confirmed the benefit of the 1μm PET media compared to the 1.2μLP media with respect to reduced retention of debris and hemoglobin, as evidenced by the lack of a requirement to rinse between units with the 1μm PET filter disposable. These results also demonstrate the feasibility of two unit washing on series with the same filter. While the current disposable set does not have bags for a second unit installed, the console and software can accommodate such a disposable configuration with minimal adjustments.

Effects of Unit Size on Performance

Testing was performed by controlling the size of the thawed frozen unit of blood being processed. Figure 11 shows the results of this testing.

The observed data indicated that as larger units are put into the filter, recovery decreases and post sup hgb and hgb released increase. Therefore, optimization of the filter to correspond with the distribution of NBRL unit sizes should include increasing the volume of the filter circuit. The current NBRL standard

operating procedure (SOP) for glycerolizing, freezing, thawing, and deglycerolizing blood calls for a range of cellular volumes from 99 to 246 milliliters (ml). The processing capacity of the filter may be increased to handle larger volumes by either increasing the volume of the filter or connecting a continuous fluid circuit for processing.

Determination of Filter Configuration for Maximizing Concentration Ability

Given that the volume of red blood cells is variable from unit to unit, an additional future feature of the TBPS should be able the ability to concentrate the variable blood volumes to a predetermined hematocrit between 70 and 80. In a preliminary evaluation of the ability of the TBPS disposable to concentrate, a method of recirculating the blood during processing was investigated. The recirculation allowed for concentration of variable units by adding flexibility to the volume of the system, unlike the current system, in which the volume is relatively fixed. The flexible volume configuration minimizes the exposure of the cells to shear by keeping them at a low hematocrit during washing and then raising the hematocrit during concentration. The recirculation configuration was tested 14 times with non-rejuvenated frozen units from NBRL (Table 44). The maximum hematocrit achieved was 78, and the recoveries using the waste hgb averaged 83%. These results show that recirculation can concentrate with an acceptable recovery.

The phases used for processing were pre-dilution, filling, washing/recirculation, and final concentration. Figure 12 illustrates the configuration of the filter disposable for recirculation. In pre-dilution, 50 mL of 12% saline is added to the thawed blood and 100 and 150 mL aliquots of processing solution are added according to the NBRL SOP as always. During filling, the line between the product bag and the pump is closed, and the filter and product bag are filled with blood and processing solution at a 2:1 ratio. After the thawed blood bag is empty, the line between the product bag and pump is opened and washing and recirculation begins. After the processing solution runs out, the final concentration phase of the washing begins. The product is still pumped through the filter and the blood is concentrated to a hematocrit of at least 70.

In order to concentrate to high hematocrits, an average of 1 1/2 units of blood were used per disposable. The use of a large amount of blood was necessitated by the need to achieve a hct greater than 70 in a filter volume of approximately 240 mL. Using large amounts of blood for processing increased the processing time and decreased the ratio of the amount of saline used per amount of blood used. The increase in processing time and decrease in proportionate amount of saline used raised the post sup hgb levels. The average post sup hgb level was 1600 mg/dL.

Subsequent testing of recirculation will use modified filters that have a smaller volume (approximately 140 mL versus approximately 240 mL for the unmodified filter) allowing for the use of normal size units of blood. The processing times will decrease and the ratio of saline to blood used will rise. Also, the 1.0 μm Cyclopore PET will be used in future testing.

Blood Supply and Degradation

During the course of development of the TBPS we have received rejuvenated and non-rejunevated frozen units from the NBRL and other blood collection sites. The rejuvenated blood was compared to the non-rejuvenated blood previously acquired from NBRL. The quality of the average rejuvenated blood unit was notably lower, with the pre-wash supernatant hemoglobin averaging 736 mg/dL (n = 35) for non-rejuvenated and 2195 mg/dL (n = 57) for rejuvenated. A summary of the data is found in Table 45. These

results demonstrated that the quality of the blood product presented for washing post-thaw was significantly poorer quality when the blood was rejuvenated before freezing as compared to frozen fresh.

During some routine testing, it was discovered that the washed unit is subject to an increase in post supernatant hemoglobin if allowed to age prior to separation of the supernatant and RBCs. In accordance with practice at NBRL, post-wash samples were separated following completion of all washes in the pool. A time study of pre-wash and post-wash blood degradation was performed. The results are available in Figures 13 and 14 and Table 45. The pre-wash units were not found to deteriorate significantly over the time required to complete processing of all units in a pool. The post-wash units indicated an elevated post sup hgb, therefore the supernatants were subsequently separated at the completion of each wash to best show the performance of our disposable and process. This tendency for degradation of the quality of the washed unit has raised questions about storage stability and this critical aspect will be investigated by Dr. Meryman at a future time.

Internal Filter Pressure Effect on Volume and Hematocrit

We have discussed previously the fact that while the filter disposable has a fixed volume in the absence of pressure within the filter. To evaluate the effect of transmembrane pressure on outlet hematocrit we examined past results. Figure15 illustrates the dependence of filter volume upon internal filter pressure. The graph was created by plotting the volume of blood removed from filters at different pressures. The filter expands at a rate proportional to the internal filter pressure.

Since the filter contains a fixed volume of RBCs, the greater the filter volume, the lower the hematocrit. Thus high pressure runs tend to yield lower hematocrits for a given input of RBC volumes. This result may explain some of the variability we have observed in outlet hematocrits for similar blood volumes.

Historical Progress Review of TBPS Disposable Development

Having achieved a process which showed the long sought improvement in post-supernatant hemoglobin it is appropriate to evaluate the improvements in performance over the course of the project. The recovery by waste supernatant hemoglobin and post supernatant hemoglobin levels for seven previous configurations have been compared statistically to results with the current process. The results from the following conditions and time periods were compared to the current averages, 86.2% recovery and 213 mg/dl post supernatant hemoglobin (manual prototype) in Table 46. The recovery and post- supernatant for the current configuration with washing performed by the automated console are statistically equivalent to the manual console.

These results (Table 46) clearly demonstrate the improvement in recovery and reduction in post-supernatant hemoglobin over the course of development.

TBPS System Development

The TBPS hardware consists of three major components: the disposable filter interface, the fluid flow path, and the console. Development of the three major components and the software for the console has been completed. Each major component, including the software, will be presented and discussed.

Filter Rotor Drive

The motor is coupled to the magdrive which drives the rotor inside the filter, which in turn washes the blood being processed. The motor and motor controller used in the magnetic drive consist of a Reliance Electric (Robbins Meyers) 12 Volt DC servo motor with feedback coming from an encoder. The motor maintains 75 ounce inches (oz-in) of torque at all times and may be controlled at speeds up to 2500 revolutions per minute (rpm). The motor is manufactured in compliance with US FDA GMP requirements, and in testing, has proven to have a long life and high reliability. The motor is relatively light and quiet, and it is easily mounted in a bracket welded into the console with a bearing to seal the motor from the outside. The encoder provides for simple feedback control from the servo amps.

The magnetic drive coupler (magdrive) is used to indirectly couple the motor to the rotor of the filter. The ingenious aspect of the coupler is that it does not require the magnetic drive of the motor to directly attach to the magnetic drive of the rotor inside the filter. This is one of the primary aspects that ensure sterility for the TBPS. The filter remains closed and the driving and driven magnets couple by magnetic force through the Acrylonitrile/Butadiene/Styrene (ABS) casing of the filter. The magdrive attaches directly to the shaft of the motor with a brass key and is locked down with a spindle that aligns the filter with the magdrive. The magdrive consists of a cylinder that has six magnetic poles inserted, three North and three South alternating. The filter contains a coated magnet that contains corresponding poles, three North and three South. The magnet in the filter is attached to the rotor. When the filter is placed in the magnetic drive coupler, the spindle aligns the filter to the center, and the magnet of the filter will align to opposite poles of the magnetic drive coupler. The magnetic force between the coupler and rotor is strong enough to allow the motor to turn the rotor without breaking the coupling, allowing for indirect control of the rotor.

Disposable Filter Interface

The interface with the disposable filter includes the magnet coupled drive for the rotor and the tubing connections between the filter and any external bags. The magnet drive consists of a motor, motor controller, and magnetic drive coupler. The tubing connections made are between the filter and waste bag, filter and processed blood bag, filter and thawed/diluted blood bag, filter and process saline solution bag, and filter and sterile vent port.

Fluid Circuit

Figure 16 is a diagram of the tubing attachments to the filter. Cyclohexanone is used as a solvent to attach the tubing to the filter and connectors to the tubing (all of which are EtO sterilized). Sterile 0.22 micron hydrophobic filters are used to create sterile barriers for the pressure tap and vent port, while sterile 0.22 micron hydrophilic filters are used to create sterile barriers for the saline solution spikes. The processed blood bag, is connected to the filter with Luer connectors. The feed tubing line (thawed/diluted blood bag, saline solution bags) is run through the pump, and a pressure tap is taken off at the mixing chamber in

order to determine the pressure inside the filter. A vent port is located at the top of the filter to allow for complete draining.

The fluid flow path is determined by the fluid schematic and the sequence of events. The fluid schematic provides for automated delivery of all solutions involved in the processing. The sequence of events lays out the fluid cycle to allow for the processing of the thawed blood (Table 1). Appendix D describes the assembly and installation of the disposable set.

The fluid schematic, presented in Figure 17, demonstrates automatic delivery of the diluents to the blood bags prior to filtration. The blood is automatically delivered to the filter along with processing solution and the processed blood is automatically delivered to the processed blood bag after processing. The schematic also allows for the automatic delivery of waste to the waste bag during processing.

The TBPS utilizes valves that are simple two-way units, allowing the valves to be solenoid pinch valves for minimized disposable cost. The pinch valves are also isolated from the fluid by acting on the exterior surface of the tubing in order to occlude the flow. Also, the placement of the valves in the schematic were carefully considered in order to reduce the number of valves used (also reducing cost). The pump is located so that it pumps both into and out of the thawed blood bags, allowing for the precise metering of diluents to the blood. The strategic location of the pump allows the system to be automated and operational with minimal incorporated equipment.

The TBPS utilizes sterile docking devices between the blood bags and the feed tubing as requested. A pressure sensor, located at the mixing chamber, is provided to record pressure data on the filter. A hemolysis sensor and glycerol sensor are placed on the waste line with a hematocrit sensor on the processed blood line.

Console Hardware

The console hardware includes the hardware components, man/machine components, monitoring devices and software design. The hardware components include the pump, pinch valves, flow meter and shaker (also the motor and magnetic drive which has already been discussed in the disposable filter interface section). The man/machine components include the display, printer, keyboard, keypad, and bar code reader. The monitoring devices include the hemolysis (hemoglobin), glycerol and pressure sensors, and bubble detector sensors. The software design includes the microprocessor, programming code, control boards and power board. The software validation is presented in Appendix E.

The general concept instrument hardware layout is shown in Figures 18 and 19. The instrument is arranged to provide maximum user access with minimum space usage. The unit's internal workings are easily accessible through the side panel. All access to user functions is done from the front, top and right sides of the unit. The user interface consists of a keyboard for data entry, a bar code scanner for data entry, a keypad for user control, a display for user notification, and a printer for data storage. The display and keypad are located on the front panel of the instrument for easy access and the bar code scanner is easily accessed on the upper right panel. The keyboard is located on a folding shelf to protect the keyboard from damage and to save space. The printer is a small stand alone unit sitting next to the unit connected by RS 232 cable.

The pump is used to meter and deliver all fluids to the bags and disposable filter. The pump includes the pump head, pump motor, encoder, and servo amp. The encoder and servo amp allows for precise metering, eliminating the need for a flow meter. The pump used is a Minntech Renal Systems peristaltic pump

system. The pump can occlude 0.06 wall tubing and has a variable flow rate between 50 to 500 ml/min. Loading tubing is simple and easy to do, while the occlusion is adjustable. The pump is quiet and, in testing, has proven to have a long life and high reliability. The pump is manufactured in compliance with US FDA GMP requirements and is able to handle multiple tubing assemblies. The pump has the added feature of having an interlock mechanism on the pump door for user safety which has been utilized.

The pinch valves are used to control the direction of flow throughout the system. The pinch valves include a solenoid, valve head, cylinder, and occluder. The solenoid receives a stroke signal to actuate the valve and occlude the tubing. The valve is easily controlled and acts only on the external surface of the tubing. The shaker attached to the console is a laboratory type shaker or upward wrist action shaker, similar to an Eberbach shaker or Burrel wrist action shaker, and it is used to dilute the thawed blood prior to processing. The shaker oscillates at approximately 180 oscillations per minute only during the dilution steps and is turned off at all other times (especially during equilibration).

A cooling fan is required to cool the electronics inside the casing of the unit. The cooling fan is a single unit that only requires power when the main unit power switch is turned on. The fan is able to cool 5 cubic feet of space 10 degrees Fahrenheit. The fan is easily mounted with four screws into the bottom of the console and it meets US FDA GMP requirements.

The display on the console is a vacuum fluorescent 4 line by 20 character display. The pressure, pump flow rate, rotor speed, glycerol level, hemoglobin level, sequence number, and sequence time are displayed. The unit alerts the user when any type of failure occurs and a message is displayed detailing the failure. For example, if the pump door is opened during processing, the unit alerts the user when "Pump Door Open" is displayed. The user runs the machine by pressing keys on the sealed membrane keypad.

The system developed for measuring hemolysis determines the amount of free hemoglobin in the waste line. After many technologies were investigated and evaluated, an optical system to measure the light absorption at a wavelength of 805 nanometers (nm) in the waste stream was chosen to measure the free hemoglobin. The waste stream tubing is sandwiched between two plates (1/2 inch thick) that aid in the determination of the absorption. An 805 nm diode is directed at the tubing. The hemoglobin concentration is calculated using the intensity of the passed light along with the length of the optical path and the absorptivity factor of hemoglobin. In order to reduce costs and conserve space, the glycerol sensor is combined with the hemoglobin sensor. The glycerol causes a change in the speed of sound, and this is utilized to determine the glycerol concentration. The ultrasound sensors are used to determine the speed of sound in the waste, which is directly correlated to the glycerol level.

Software for the unit has been developed in general accordance with ANSI / IEEE standard 730-1989 as detailed in the IEEE Standards Collection, Software Engineering, 1993 edition. the SQAP, SRS, SDD SVP, and SUR have all been completed (see Appendix E).

Conclusions

AHT has completed Phase II SBIR development of its Thawed Blood Processing System (TBPS). The goals of the development are outlined earlier in this report. A summary of the accomplishment of these Technical Objectives is listed in Tables 47 and 48 for the disposable filter and the console, respectively.

Reviewing the accomplishments, all essential hardware console Technical Objectives were met (Table 48). The software validation (Appendix E) has certified that the console hardware does perform program tasks.

Items which were not incorporated in the console and deemed non-essential for this phase were the switchable 110/220 power supply, warning display, power failure detector, measurement of outlet hematocrit and incorporation of the shaker and printer into the console unit. All of these elements could be incorporated if development of the TBPS continues.

The performance of the TBPS for washing blood has improved significantly during the course of development (Table 46). A number of disposable configurations have been evaluated. Reviewing the progression of development it is clear that the filter media is a major determinant of performance. Initially, based on Phase I studies, the Pall Loprodyne (LP) 1.2μm media was selected. A second determinant of performance was the rotor type and speed. Utilizing the Pall 1.2μm LP media disposable, a modification of the rotor was required to improve mixing to reduce adherence of cells and stroma on the membrane media. After exhausting modifications to disposable configuration utilizing the Pall 1.2μm LP media disposable, we reevaluated filter medias. Results from this evaluation demonstrated the primary dependence on media type with respect to TBPS performance. From the media reevaluation, the Whatman 1μm PET media was selected. Further optimization of the disposable configuration and integration with the automated console lead to the final design.

The TBPS washed blood product has the following major characteristics:

- glycerol washout <1%
- recovery >80% if post-thaw recovery >90%
- supernatant hgb levels typically between 150-300mg/dL
- hematocrit of 60-75%
- some degradation of quality over 24 hour period as evidenced by increased in supernatant hgb

The Technical Objectives for the blood product have been met with respect to glycerol washout, hematocrit and recovery. Supernatant hgb levels immediately post-wash and after 24 hours have been the major shortcoming of the TBPS. We have demonstrated during the course of development that the ability to remove free hgb from the disposable is dependent on the media used, the mixing by the rotor, the relative amount of shear from the rotor and the ability of stroma to pass through the filter media. A major improvement has been made during development in the post-wash supernatant hgb levels. The current post-wash supernatant hgb levels are near our initial objective and appears to be dependent on the quality of the blood being washed. An issue which remains to be addressed is the hemolysis of the washed blood post-wash. It is envisioned that through the use of additive solutions this degradation can be limited. We look forward to evaluation of this issue by Dr. Meryman's laboratory.

The disposable tubing and assembly has been completed and meets the majority of the initial Technical Objectives. The disposable has been developed to utilize a single pump compared to multiple fluid control devices. Sterile disposables, while not validated, should be readily validated based on the validation of a comparable disposable.

In conclusion, AHT has met the essential element of the TBPS development plan and demonstrated significant improvement in the quality of blood product washed with the TBPS as well as completed the design, assembly and validation of an automated console to process frozen thawed blood.

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List of Tables

Table 1: Sequence of Events for TBPS Console

Event #	Description	Pumped Amount (ml)	Pump Rate (ml/min)	Rotor	Shaker	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
1	Process Start															
2	Dilution of Thawed Blood Unit #1 with 12% Saline Solution	50	100		X			X		X						
3	Equilibration of Unit #1 for 2 minutes	0	0													
4	Dilution of Thawed Blood Unit #1 with 0.9% Saline/0.2% Glucose Solution	100	100		X				X	X						
5	Equilibration of Unit #1 for 2 minutes	0	0													
6	Dilution of Thawed Blood Unit #1 with 0.9% Saline/0.2% Glucose Solution	150	100		X				X	X						
7	Equilibration of Unit #1 for 2 minutes	0	0													
8	Priming of Filter with 0.9% Saline/0.2% Glucose Solution	300-400	100						X			X		X		
9	Spin-Up of Filter Rotor	0	0	X										X		
10	Pumping of Diluted Unit #1 into Filter	1300	100	X		X						X		X		
11	Pumping of 0.9% Saline/0.2% Glucose Solution into Filter	Note 1	100	X					X			X		X		
12	Spin-Down of Filter Rotor	0	0													
13	Draining of Filter to Washed Unit #1	0	0										X		X	
14	Process End															

NOTES 1) Delivery Continues Until S4 Threshold Obtained
2) Refer to Figure * for V1-V11 and S4
3) X = Actuation of Valves, Shaker, and/or Rotor
4) Rotor Speed when Actuated is 800 RPM

Table 2a: Sample Calculation of Blood Volume for BUP I				
Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC to be Washed (ml)
214	40	85.6	20	428
214	50	107	20	535
214	60	128.4	20	642
214	70	149.8	20	749

Table 2b: Sample Calculation of Blood Volume for WUP				
Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC to be Washed (ml)
518	40	207.2	20	1036
518	50	259	20	1295
518	60	310.8	20	1554
518	70	362.6	20	1813

Table 3: Sample Calculation of Blood Volume for BUP I				
Internal Volume of Filter (ml)	Target Final hct in Filter (%)	Volume of RBCs (ml)	Initial hct of RBC for Washing (%)	Volume of RBC Suspension to be Washed (ml)
214	40	92	20	458
214	50	114	20	572
214	60	137	20	687
214	70	160	20	801
280	40	120	20	599
280	50	150	20	749
280	60	180	20	899
280	70	210	20	1049
320	40	137	20	685
320	50	171	20	856
320	60	205	20	1027
320	70	240	20	1198

Net Weight of RBC Unit Before Freezing (hct=75%)	Net volume of RBC Unit Before Freezing (hct=75%)
150	140
200	187
220	206
240	224
300	280
330	308

Table 4: Effect of Target Hct on 214 Plain Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Target Hct. 55 or less									
28	15.3	100	1200	73	42.1	48.0	114.1	82	263
29	12.5	120	1200	76	42.1	48.0	114.1	334	
30	10.8	140	1200	76	42.1	48.0	114.1	334	160
25	14.0	100	1200	69	46.5	54.0	116.1	60	124
27	12.5	120	1200	90	50.5	55.0	109.0		186
26	10.7	120	1200	83	52.7	58.0	110.0		391
21	15.8	100	1200	86	54.7	58.4	106.8	88	459
22	15.0	100	800	333	54.7	48.9	89.4	88	201
24	15.8	100	1200	75	55.0	62.0	112.8	60	
AVG	13.6		1155.6	106.8	48.9	53.4	109.6	149.4	254.9
SD	2.0		133.3	85.1	5.8	5.4	8.1	126.6	125.2
(B) Target Hct. 55 - 65									
19	12.8	100	1200	113	58.6	61.0	104.1		
20	10.4	150	1200	195	58.6	58.8	100.3		256
56	16.1	100	1200	108	58.6	54.8	93.5	806	168
48	16.0	100	1200	79	58.9	54.6	92.6	444	1154
49	16.8	100	1200	78	58.9	57.1	96.9	444	699
53	16.0	100	1500	433	58.9	56.8	96.4	67	419
50	16.0	100	1200	252	59.6	54.0	90.6	67	522
51	16.0	100	1200	173	59.6	57.5	96.4	67	578
52	16.0	100	1200	132	59.6	58.1	97.4	67	541
44	16.5	100	1200	92	59.9	61.7	103.1	5	652
45	16.5	100	1200	295	59.9	51.8	86.5	5	221
46	16.3	100	1200	77	62.0	56.3	90.8	5	132
47	16.0	100	1200	108	62.0	55.4	89.4	5	443
23	16.0	100	1200	134	63.4	67.0	105.6	60	451
83	12.0	100	1200	114	64.0	59.7	93.2		
AVG	15.3		1220.0	158.9	60.2	57.6	95.8	170.2	479.7
SD	1.9		77.5	99.5	1.8	3.7	5.6	255.5	272.1
(C) Target Hct. 66 - 80									
82	12.0	100	1200	157	65.6	56.4	85.9		
81	13.0	100	1200	167	66.5	56.7	85.2		
79	14.0	100	1200	94	67.0	54	80.5		
80	15.0	100	1200	156	67.1	55.3	82.4		
36	25.0	100	1200	267	67.4	60.0	89.0	49	337
18	16.8	100	1200	199	67.6	65.0	96.1	61	662
39	16.8	100	1200	311	68.3	58.6	85.7	92	453
57	16.3	100	1500	99	68.7	55.5	80.8	800	376
58	17.3	100	1500	219	68.7	55.9	81.4	800	376
176	17	100	1100	514	68.7	58.9	85.8	37	144
178	17	100	1100	401	68.7	59.4	86.5	37	83
59	17.5	100	1200	224	70.4	54.7	77.7	800	668
42	16.3	100	1200	288	72.9	57.9	79.4	220	753
37	24.0	120	1200	380	74.6	61.3	82.2		399
60	17.5	100	1200	356	76.7	64.5	84.1	205	740
61	26.0	50	1200	521	77.1	60.5	78.5	205	249
43	16.8	100	1200	397	77.8	59.3	76.3	220	375
AVG	17.5		1223.5	279.4	70.2	58.5	83.4	293.8	431.9
SD	3.9		109.1	132.8	4.0	3.2	4.8	313.6	217.0
(D) Target Hct. > 80									
38	21.5	140	1200	405	81.8	62.6	76.5		373
97	16.5	100	1200	271	84.8	55.4	65.3		
77	22.0	100	1200	433	86.1	54.5	63.3		
78	18.5	100	1200	382	87.5	52.3	59.7		
91	18.0	100	1200	307	87.0	52.0	59.8		
94	16.0	100	1200	321	87.1	49.0	56.2		
AVG	18.8		1200.0	353.2	85.7	54.3	63.5		373.0
SD	2.5		0.0	62.9	2.2	4.6	7.1		

Table 5 : Marker Washout Results for Ca - Doped Fresh RBC Washed w/ 214 Plain Filters

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Hct. Recovery	Calcium Washout (%)
18	16.8	100	1200	199	96.1	98.95
19	12.8	100	1200	113	104.1	98.83
20	10.4	150	1200	195	100.3	98.76
21	15.8	100	1200	86	106.8	97.61
22	15.0	100	800	333	89.4	97.06
23	16.0	100	1200	134	105.6	99.45
24	15.8	100	1200	75	112.8	99.36
25	14.0	100	1200	69	116.1	99.23
26	10.7	120	1200	83	110.0	98.93
27	12.5	120	1200	90	109.0	98.87
28	15.3	100	1200	73	114.1	98.31
29	12.5	120	1200	76	114.1	98.31
30	10.8	140	1200	76	114.1	98.31
36	25.0	100	1200	267	89.0	99.08
37	24.0	120	1200	380	82.2	98.96
38	21.5	140	1200	405	76.5	99.28
39	16.8	100	1200	311	85.7	99.51
42	16.3	100	1200	288	79.4	99.33
43	16.8	100	1200	397	76.3	99.35
44	16.5	100	1200	92	103.1	99.31
45	16.5	100	1200	295	86.5	99.28
46	16.3	100	1200	77	90.8	85.19
47	16.0	100	1200	108	89.4	99.33
48	16.0	100	1200	79	92.6	99.27
49	16.8	100	1200	78	96.9	99.31
50	16.0	100	1200	252	90.6	99.30
51	16.0	100	1200	173	96.4	99.35
52	16.0	100	1200	132	97.4	99.36
53	16.0	100	1500	433	96.4	99.08
56	16.1	100	1200	108	93.5	98.17
57	16.3	100	1500	99	80.8	98.19
58	17.3	100	1500	219	81.4	98.21
59	17.5	100	1200	224	77.7	98.16
60	17.5	100	1200	356	84.1	98.57
61	26.0	50	1200	521	78.5	99.28
77	22.0	100	1200	433	63.3	96.44
78	18.5	100	1200	382	59.7	96.65
AVG	17		1214	208	93	98
SD	4		108	135	14	2

Table 6 : Effect of Transmembrane Pressure on 214 Plain Filter Performance for Washed Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Maximum Transmembrane Pressure < 300									
25	14.0	100	1200	69	46.5	54.0	116.1	60	124
28	15.3	100	1200	73	42.1	48.0	114.1	82	263
24	15.8	100	1200	75	55.0	62.0	112.8	60	
29	12.5	120	1200	76	42.1	48.0	114.1	334	
30	10.8	140	1200	76	42.1	48.0	114.1	334	160
46	16.3	100	1200	77	62.0	56.3	90.8	5	132
49	16.8	100	1200	78	58.9	57.1	96.9	444	699
48	16.0	100	1200	79	58.9	54.6	92.6	444	1154
26	10.7	120	1200	83	52.7	58.0	110.0		391
21	15.8	100	1200	86	54.7	58.4	106.8	88	459
27	12.5	120	1200	90	50.5	55.0	109.0		186
44	16.5	100	1200	92	59.9	61.7	103.1	5	652
79	14.0	100	1200	94	67.0	54	80.5		
57	16.3	100	1500	99	68.7	55.5	80.8	800	376
47	16.0	100	1200	108	62.0	55.4	89.4	5	443
56	16.1	100	1200	108	58.6	54.8	93.5	806	168
19	12.8	100	1200	113	58.6	61.0	104.1		
83	12.0	100	1200	114	64.0	59.7	93.2		
52	16.0	100	1200	132	59.6	58.1	97.4	67	541
23	16.0	100	1200	134	63.4	67.0	105.6	60	451
80	15.0	100	1200	156	67.1	55.3	82.4		
82	12.0	100	1200	157	65.6	56.4	85.9		
81	13.0	100	1200	167	66.5	56.7	85.2		
51	16.0	100	1200	173	59.6	57.5	96.4	67	578
20	10.4	150	1200	195	58.6	58.8	100.3		256
18	16.8	100	1200	199	67.6	65.0	96.1	61	662
58	17.3	100	1500	219	68.7	55.9	81.4	800	376
59	17.5	100	1200	224	70.4	54.7	77.7	800	668
50	16.0	100	1200	252	59.6	54.0	90.6	67	522
36	25.0	100	1200	267	67.4	60.0	89.0	49	337
97	16.5	100	1200	271	84.8	55.4	65.3		
42	16.3	100	1200	288	72.9	57.9	79.4	220	753
45	16.5	100	1200	295	59.9	51.8	86.5	5	221
AVG	15.2		1218.2	143.0	60.5	56.5	95.2	246.2	440.5
SD	2.7		72.7	71.9	9.2	4.2	12.8	292.8	247.0
(B) Maximum Transmembrane Pressure > 301									
91	18.0	100	1200	307	87.0	52.0	59.8		
39	16.8	100	1200	311	68.3	58.6	85.7	92	453
94	16.0	100	1200	321	87.1	49.0	56.2		
22	15.0	100	800	333	54.7	48.9	89.4	88	201
60	17.5	100	1200	356	76.7	64.5	84.1	205	740
37	24.0	120	1200	380	74.6	61.3	82.2		399
78	18.5	100	1200	382	87.5	52.3	59.7		
43	16.8	100	1200	397	77.8	59.3	76.3	220	375
178	17	100	1100	401	68.7	59.4	86.5	37	83
38	21.5	140	1200	405	81.8	62.6	76.5		373
53	16.0	100	1500	433	58.9	56.8	96.4	67	419
77	22.0	100	1200	433	86.1	54.5	63.3		
61	26.0	50	1200	521	77.1	60.5	78.5	205	249
176	17	100	1100	514	68.7	58.9	85.8	37	144
AVG	18.7		1178.6	392.4	75.4	57.0	77.2	118.9	343.6
SD	3.3		142.4	67.4	10.5	5.0	12.6	78.2	187.6

Table 7: BUP I Evaluation of the Effect of Inputting Blood and Saline Simultaneously

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Calcium Washout (%)	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
47 B-S	16.0	100	1200	108	62.0	55.4	89.4	98.77	5	443
46 Sim	16.3	100	1200	77	62.0	56.3	90.8	72.30	5	132
48 B-S	16.0	100	1200	79	58.9	54.6	92.6	98.68	444	1154
49 Sim	16.8	100	1200	78	58.9	57.1	96.9	98.68	444	699

Table 8: Performance of 214 Plain Filter for Washing Frozen Thawed Cells

Name	Rotor Speed	Nominal Flow Rate	Max. Pressure	Thaw / Dilute Recovery	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Post-Dilute - Weight	Post-Wash - Weight	Post-dilute Sup Hb	Post -wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Osm.	Waste volume	Intra K+ mEg/ml
Brenda Mayer	1200	100	56	97	81.8	55.7	550	180	348	288	388	49.5	296	1599	
David Mayer	1200	100	57	91.7	69.8	47.4	500	170	1077	280	689	44	298	1548	
Joe Caroll	1200	100	362	95.4	69.4	44.4	655	123	466	901	523	63	314	1670	
Paul Carroll	1200	100	202	93.5	60.2	28.4	675	210	1322	717	850	56	314	1730	
P. Pitt	1200	100	363	96.1	71.9	78.4	660	306	554	760	685	56	336	2930	
T. Pitt	1200	100	251	96	76	73.5	710	287	597	460	685	49	310	2113	
S. Jones	1000	100	47	95.9	73.9	55.1	433	183	484	274		36.5	307	1619	4.07
B. Miller	1200	100	104	96.1	77.6	63.4	615	178	356	395		56	298	1673	3.67
B. Jones	1200	100	107	98.1	84.8	80.3	570	217	202	395		58	301	1767	4.27
Schwinger	1200	100	60	97.5	71.6	49.9	427	187	447	401	616	50	299	1475	4.87
Lee	1200	100	230	97.6	82.5	76.7	590	236	278	727	529	60	354	1388	4.27
Miller	1200	100	72	95.4	61.4	43	529	188	752	544	894	49	313	1802	4.48
AVG	1183.3		159.3	95.9	73.4	58.0	576.2	205.4	573.6	511.8	651.0	52.3	311.7	1776.2	4.3
SD	57.7		118.6	1.8	7.7	16.5	92.5	50.9	331.1	214.3	159.7	7.4	17.4	406.7	0.4

Table 9 : Effect of Rotor Modification on 214 Filter Performance for washing Fresh Blood Cells

Test #	Filter Type	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
91	214 pln	18.0	100	1200	307	87.0	52.0	59.8
94	214 pln	16.0	100	1200	321	87.1	49.0	56.2
97	214 pln	16.5	100	1200	271	84.8	55.4	65.3
AVG		16.8		1200.0	299.7	86.3	52.1	60.4
93	214 3R	17.0	100	900	398	87.1	48.2	55.3
95	214 3R	17.0	100	800-1100	303	84.8	51.0	60.1
AVG		17.0		900.0	350.5	86.0	49.6	57.7
96	214 3R 2H	15.5	100	1000	220	84.8	59.5	70.2
127	214 12R 6H	20.8	100	900	192	80.8	66.6	82.4
132	214 12R 6H	17.0	100	900	206	75.5	68.2	90.3
133	214 12R 6H	17.0	100	900	283	80.5	65.1	80.9
134	214 12R 6H	17.0	100	700	224	75.1	65.7	87.4
135	214 12R 6H	18.0	100	700	376	80.5	62.1	77.2
AVG		18		820	256	78	66	84

Table 10: Effect of Increasing Flowrate on Performance with a 214 12R6H Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
102	11.5	100-200	900	145	58.5	50.0	85.4
104	11.5	100-150	900	128	58.5	60.3	103.0
AVG	11.5		900.0	136.5	58.5	55.2	94.2
SD	0.0		0.0	12.0	0.0	7.3	12.4

Table 11 : Effect of Target Hct on 214 12R6H Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Target HCT 55 - 70%									
102	11.5	100-200	900	145	58.5	50.0	85.4	309	801
103	10.5	150	900	143	58.5	58.1	99.3	309	825
104	11.5	100-150	900	128	58.5	60.3	103.0	309	795
105	10.5	150	800	136	58.5	58.3	99.6	309	395
106	14.5	100	800	94	63.1	58.1	92.0	306	694
107	9.5	150	900	118	63.1	57.9	91.7	306	398
125	16.3	100	900	108	64.6	61.1	94.6		
128	15.0	100	900	117	64.7	62.5	96.6		
114	18.0	100	900	153	67.6	63.2	93.4		
101	15.5	100	900	133	68.4	65.8	96.2		
AVG	13.3		880.0	127.5	62.6	59.5	95.2	308.0	651.3
SD	2.9		42.2	18.3	3.9	4.3	5.0	1.5	202.4
(B) Target HCT 71 - 80%									
163	24.0	60	600	206	72.0	61.5	85.5	35	201
164	15.0	100	600	369	72.0	60.2	83.7	35	263
165	16.0	100	800	203	72.0	62.3	86.6	35	344
172	18	100	800	577	72.1	64.7	89.7	34	1170
175	17	100	800	432	73.5	63.8	86.9	37	308
161	15.0	100	600	282	73.8	56.6	76.7	16	480
162	15.0	100	800	242	73.8	62.0	84.1	16	106
144	12.0	150	800	374	75.7	60.3	79.6	461	2445
145	10.0	150	800	330	75.7	58.3	77.0	461	2399
142	11.0	150	800	350	78.3	60.9	77.8	818	2119
AVG	15.3		740.0	336.5	73.9	61.1	82.7	194.8	983.5
SD	4.0		96.6	113.7	2.1	2.4	4.6	283.1	970.9
(C) Target HCT > 80%									
134	17.0	100	700	224	80.4	65.7	81.7	331	73
127	20.8	100	900	192	80.8	66.6	82.4		
132	17.0	100	900	206	80.8	68.2	84.4	331	67
133	17.0	100	900	283	86.1	65.1	75.6	331	725
135	18.0	100	700	376	86.1	62.1	72.1	331	460
AVG	18.0		820.0	256.2	82.8	65.5	79.3	331.0	331.3
SD	1.6		109.5	75.4	3.0	2.3	5.2	0.0	320.5

Table 12: Summary Fresh Blood Washing w/ 214 12R6H Filter

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct Recovery %	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) 100ml/min Flowrate W/O Clam Shell									
106	14.5	100	800	94	60.1	58.1	96.7	306	694
128	15.0	100	900	117	61.5	62.5	101.6		
125	16.3	100	900	108	61.4	61.1	99.5		
114	18.0	100	900	153	64.4	64.0	99.4		
101	15.5	100	900	133	64.8	65.8	101.5		
127	20.8	100	900	192	76.8	66.6	86.8		
114	18.0	100	900	153	64.4	63.2	98.1		
128	15.0	100	900	117	61.5	62.5	101.6		
132	17.0	100	900	206	71.7	68.2	95.1	331	67
133	17.0	100	900	283	76.4	65.1	85.2	331	725
134	17.0	100	700	224	71.3	65.7	92.1	331	73
135	18.0	100	700	376	76.4	62.1	81.3	331	460
136	14.0	100	800	111	87.3	61.4	70.3	320	76
161	15.0	100	600	282	65.4	56.6	86.5	16	480
162	15.0	100	800	242	65.4	62.0	94.8	16	106
163	24.0	60	600	206	63.8	61.5	96.4	35	201
164	15.0	100	600	369	63.8	60.2	94.4	35	263
165	16.0	100	800	203	63.8	62.3	97.7	35	344
Average	16.7	97.8	805.6	198.3	67.8	62.7	93.3	189.7	317.2
(B) 100ml/min Flowrate With Clam Shell									
172	18	100	800	577	63.9	64.7	101.3	34	1170
175	17	100	800	432	65.0	63.8	98.2	37	308
177	17	100	600	475	65.0	60.7	93.4	37	1542
Average	17.3	100.0	733.3	494.7	64.6	63.1	97.7	36.0	1006.7
(C) 150ml/min Flowrate W/O Clam Shell									
103	10.5	150	900	143	55.5	58.1	104.7	309	825
105	10.5	150	800	136	55.5	58.3	105.0	309	395
107	9.5	150	900	118	60.1	57.9	96.3	306	398
137	12.0	150	800	145	87.3	61.2	70.1	320	885
142	11.0	150	800	350	69.5	60.9	87.6	818	2119
144	12.0	150	800	374	67.2	60.3	89.8	461	2445
145	10.0	150	800	330	67.2	58.3	86.8	461	2399
Average	11	150	829	228	66	59	91	426	1352

Table 13: Effect of Transmembrane Pressure on 214 12 R6H Plain Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) Transmembrane Pressure < 300 mmHg									
106	14.5	100	800	94	63.1	58.1	92.0	306	694
125	16.3	100	900	108	64.6	61.1	94.6		
128	15.0	100	900	117	64.7	62.5	96.6		
107	9.5	150	900	118	63.1	57.9	91.7	306	398
104	11.5	100-150	900	128	58.5	60.3	103.0	309	795
101	15.5	100	900	133	68.4	65.8	96.2		
105	10.5	150	800	136	58.5	58.3	99.6	309	395
103	10.5	150	900	143	58.5	58.1	99.3	309	825
102	11.5	100-200	900	145	58.5	50.0	85.4	309	801
114	18.0	100	900	153	68.0	64.0	94.1		
127	20.8	100	900	192	80.8	66.6	82.4		
165	16.0	100	800	203	72.0	62.3	86.6	35	344
132	17.0	100	900	206	80.8	68.2	84.4	331	67
163	24.0	60	600	206	72.0	61.5	85.5	35	201
134	17.0	100	700	224	80.4	65.7	81.7	331	73
162	15.0	100	800	242	73.8	62.0	84.1	16	106
161	15.0	100	600	282	73.8	56.6	76.7	16	480
133	17.0	100	900	283	86.1	65.1	75.6	331	725
AVG	15.3		833.3	172.9	69.2	61.3	89.4	226.4	454.2
SD	3.7		102.9	58.7	8.8	4.4	8.0	139.8	289.4
(B) Transmembrane Pressure > 300 mmHg									
145	10.0	150	800	330	75.7	58.3	77.0	461	2399
142	11.0	150	800	350	78.3	60.9	77.8	818	2119
164	15.0	100	600	369	72.0	60.2	83.7	35	263
144	12.0	150	800	374	75.7	60.3	79.6	461	2445
135	18.0	100	700	376	86.1	62.1	72.1	331	460
175	17	100	800	432	73.5	63.8	86.9	37	308
172	18	100	800	577	72.1	64.7	89.7	34	1170
AVG	14.4		757.1	401.1	76.2	61.5	81.0	311.0	1309.1
SD	3.4		78.7	83.6	4.9	2.2	6.1	297.3	997.6

Table 14: Effect of Flowrate on 214 12R6H Filter Performance for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery
(A) Flowrate <= 100							
163	24.0	60	600	206	72.0	61.5	85.5
101	15.5	100	900	133	68.4	65.8	96.2
106	14.5	100	800	94	63.1	58.1	92.0
114	18.0	100	900	153	68.0	64.0	94.1
125	16.3	100	900	108	64.6	61.1	94.6
127	20.8	100	900	192	80.8	66.6	82.4
128	15.0	100	900	117	64.7	62.5	96.6
132	17.0	100	900	206	80.8	68.2	84.4
133	17.0	100	900	283	86.1	65.1	75.6
134	17.0	100	700	224	80.4	65.7	81.7
135	18.0	100	700	376	86.1	62.1	72.1
161	15.0	100	600	282	73.8	56.6	76.7
162	15.0	100	800	242	73.8	62.0	84.1
164	15.0	100	600	369	72.0	60.2	83.7
165	16.0	100	800	203	72.0	62.3	86.6
172	18	100	800	577	72.1	64.7	89.7
175	17	100	800	432	73.5	63.8	86.9
AVG	17.0	97.6	794.1	246.9	73.7	63.0	86.0
SD	2.4	9.7	114.4	129.3	7.1	3.0	7.2
(B) Flowrate = 150 ml/min							
103	10.5	150	900	143	58.5	58.1	99.3
105	10.5	150	800	136	58.5	58.3	99.6
107	9.5	150	900	118	63.1	57.9	91.7
142	11.0	150	800	350	78.3	60.9	77.8
144	12.0	150	800	374	75.7	60.3	79.6
145	10.0	150	800	330	75.7	58.3	77.0
AVG	10.6		833.3	241.8	68.3	59.0	87.5
SD	0.9		51.6	121.0	9.3	1.3	10.7

Table 15 : Marker Washout Results for Ca Doped Fresh RBC washed w/ 214 12R6H Filter

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Hct. Recovery	Calcium Washout (%)
101	15.5	100	900	133	96.2	99.35
102	11.5	100-200	900	145	85.4	99.24
103	10.5	150	900	143	99.3	99.36
104	11.5	100-150	900	128	103.0	99.31
105	10.5	150	800	136	99.6	99.27
106	14.5	100	800	94	92.0	99.29
107	9.5	150	900	118	91.7	99.29
125	16.3	100	900	108	94.6	99.50
127	20.8	100	900	192	82.4	99.23
128	15.0	100	900	117	96.6	99.52
AVG	14		880	131	94	99
SD	3		42	27	6	0

Table 16: Comparison of Filter Performance w/ Fresh Blood as a function of Rotor Design w/214 ml Filter

Test #	Filter Type	Dilute Blood Volume In (mL)	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct Recovery %	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
(A) 214 12R 6H vs 214 Pln											
175	214 12R 6H	794	17	100	800	432	65.0	63.8	98.2	37	308
176	214 pln	794	17	100	1100	514	65.0	58.9	90.7	37	144
178	214 pln	794	17	100	1100	401	65.0	59.4	91.4	37	83
(B) 214 12R 6H vs 214 Pln 6H											
172	214 12R 6H	731	18	100	800	577	63.9	64.7	101.3	34	1170
171	214 Pln 6H	731	16	100	1100	242	63.9	58.9	92.2	34	211
173	214 Pln 6H	731	16	100	1100	300	63.9	57.4	89.9	34	339
(C) 214 12R 6H vs 214 12R											
181	214 12R-6H	638	30	100	800	444	59.7	65.7	110.1	9	1573
180	214-12R	636	30	100	800	409	59.4	67	112.8	9	1024
(D) 214 12R 6H vs 214 6B											
181	214 12R-6H	638	30	100	800	444	59.7	65.7	110.1	9	1573
179	214 6B	696	17	100	700	254	65.1	62	95.3	9	1405
(E) 214 12R 6H vs 214 6B 6H											
172	214 12R 6H	731	18	100	800	577	63.9	64.7	101.3	34	1170
170	214 6B 6H	731	23	70	700	386	63.9	60.9	95.4	34	1311
(F) 214 12R 6H vs 214 Pln 12B 6H											
175	214 12R 6H	794	17	100	800	432	65.0	63.8	98.2	37	308
177	214 12B 6H	794	17	100	600	475	65.0	60.7	93.4	37	1542

Table 17: Compilation of all Frozen Thawed Blood Testing Results for the Fourth Quarter

Unit #	Filter Type	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post-dilute Sup Hb	Post - wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Waste volume	Intra K+ mEg/ml
95-02217	214 12R-6H	1/4/96	800	100	13	294	61.9	44.8	862	1454	935	1068	43.5	1638	
95-02220	214 12R-6H	1/4/96	800	150	13	470	76.9	70.5	618	414	1158	753	55	1801	
95-02227	214 12R-6H	1/11/96	800	150	13	352			371	442	864	530	45	1963	
95-02218	214 12R-6H	1/11/96	800	150	12	267			408	661	719	566	45.5	1942	
95-02224	214 12R-6H	1/22/96	800	150	18	537	72.2	63.3	603	545	882	560	51.5	2683	4.47
95-02230	214 12R-6H	1/22/96	800	150	15	372	73.7	66.4	641	438	652	504	51	2453	4.44
	214 12R-6H	2/1/96	800	150	18	352	69.6	65		182	420	455	54	2816	4.24
8060243	214 12R-6H	2/1/96	800	150	25	251	69.8	66.7		294	882	663	51.5	2284	4.67
8091485	214 12R-6H	2/6/96	800	150	18	458	70.3	66.2		245	1158	543	49	2626	4.86
95-01585	214 12R-6H	2/8/96	800	150	15	157	75.5	65.8	697		678	410	57	2417	5.1
95-01634	214 12R-6H	2/8/96	800	150	15	396	66.3	50.8	1111		556	700	56	2373	5.51
95--02205	214 12R-6H	2/12/96	800	150	11	195	68.2	64.3	284		606	630	46.5	1931	4.87
95-01568	214 12R-6H	2/12/96	800	150	20	580	69.2	71.5	729		731	784	56	1845	4.94
1628/1631 b	214 12R-6H	2/22/96	800	100	20.0	366	73.5	73.9	560		220	555	58	2264	4.68
1628/1631a	214 12R-6H	2/22/96	600	100	20.0	252	73.9	69	2633		386	547	51	2279	4.66
1583/1616b	214 12R-6H	3/4/96	800	100	30	444	71.8	70.3	1798		522	814	65	1924	5.07
1583/1616a	214-12R	3/4/96	800	100	30	409	72.8	69.3	1798		642	763	66	1801	4.37
379/89/1411	214 6R-6H	3/7/96	800	100	38	602	73.6	76	1518		422	551	59	2439	4.55
379/89/1411	214 12R-6H	3/6/96	800	75	47	435	75.1	68.7	1518		424	470	56	2490	4.76
373/85/1429	214 12R-6H	3/7/96	800	50	32	689	74.3	71.9	1202		1049	515	64	2566	5.18
373/85/1429	214 12R-6H	3/7/96	800	50	34	678	73.9	70.1	1202		723	536	63.5	2538	4.81
373/85/1429	214 6R-6H	3/7/96	800	60	28	769	75.9	73.3	1202		630	494	62	2490	5.31
Average			790.9	119.8	22.0	423.9	71.9	66.9	1039.7	519.4	693.6	609.6	54.8	2252.9	4.8

Table 18: Effect of Operating Conditions on Performance of the 214 12R6H Filter w/ Frozen thawed cells

Unit #	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post - wash - Sup Hb	Waste Sup Hb.	Post - wash Hct	Intra K+ mEg/ml
(A) Flow rate 100 ml min or less												
95-02217	1/4/96	**800**	**100**	13	294	61.9	44.8	862	935	1068	43.5	
1628/1631 b	2/22/96	**800**	**100**	20.0	366	73.5	73.9	560	220	555	58	4.68
1583/1616b	3/4/96	**800**	**100**	30	444	71.8	70.3	1798	522	814	65	5.07
1379/89/1411a	3/6/96	**800**	**75**	47	435	75.1	68.7	1518	424	470	56	4.76
1373/85/1429a	3/7/96	**800**	**50**	32	689	74.3	71.9	1202	1049	515	64	5.18
1373/85/1429b	3/7/96	**800**	**50**	34	678	73.9	70.1	1202	723	536	63.5	4.81
Average					484.3	71.8	66.6	1190.3	645.5	659.7	58.3	4.9
(B) Flow rate												
95-02220	1/4/96	**800**	**150**	13	470	76.9	70.5	618	1158	753	55	
95-02227	1/11/96	**800**	**150**	13	352			371	864	530	45	
95-02218	1/11/96	**800**	**150**	12	267			408	719	566	45.5	
95-02224	1/22/96	**800**	**150**	18	537	72.2	63.3	603	882	560	51.5	4.47
95-02230	1/22/96	**800**	**150**	15	372	73.7	66.4	641	652	504	51	4.44
	2/1/96	**800**	**150**	18	352	69.6	65		420	455	54	4.24
8060243	2/1/96	**800**	**150**	25	251	69.8	66.7		882	663	51.5	4.67
8091485	2/6/96	**800**	**150**	18	458	70.3	66.2		1158	543	49	4.86
95-01585	2/8/96	**800**	**150**	15	157	75.5	65.8	697	678	410	57	5.1
95-01634	2/8/96	**800**	**150**	15	396	66.3	50.8	1111	556	700	56	5.51
95--02205	2/12/96	**800**	**150**	11	195	68.2	64.3	284	606	630	46.5	4.87
95-01568	2/12/96	**800**	**150**	20	580	69.2	71.5	729	731	784	56	4.94
Average					365.6	71.2	65.1	606.9	775.5	591.5	51.5	4.8

Table 19: Effect of Wash Volume on performance of 214 12R6H Filter w/ Frozen Thawed Cells

Unit #	Date of Test	Waste volume	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post - wash - Sup. Hb	Waste Sup Hb.	Intra K+ mEg/ml
(A) Waste Volume < 2000 ml										
95-02217	1/4/96	**1638**	13	294	61.9	44.8	862	935	1068	
95-02220	1/4/96	**1801**	13	470	76.9	70.5	618	1158	753	
95-02227	1/11/96	**1963**	13	352			371	864	530	
95-02218	1/11/96	**1942**	12	267			408	719	566	
95--02205	2/12/96	**1931**	11	195	68.2	64.3	284	606	630	4.87
95-01568	2/12/96	**1845**	20	580	69.2	71.5	729	731	784	4.94
1583/1616b	3/4/96	**1924**	30	444	71.8	70.3	1798	522	814	5.07
Average		**1863.4**	16.0	371.7	69.6	64.3	724.3	790.7	735.0	5.0
(B) Waste Volume 2000 - 2500 ml										
95-02230	1/22/96	**2453**	15	372	73.7	66.4	641	652	504	4.44
8060243	2/1/96	**2284**	25	251	69.8	66.7		882	663	4.67
95-01585	2/8/96	**2417**	15	157	75.5	65.8	697	678	410	5.1
95-01634	2/8/96	**2373**	15	396	66.3	50.8	1111	556	700	5.51
1628/1631 b	2/22/96	**2264**	20.0	366	73.5	73.9	560	220	555	4.68
1628/1631a	2/22/96	**2279**	20.0	252	73.9	69	2633	386	547	4.66
379/89/1411	3/6/96	**2490**	47	435	75.1	68.7	1518	424	470	4.76
373/85/1429	3/7/96	**2490**	28	769	75.9	73.3	1202	630	494	5.31
Average		**2381.3**	23.1	374.8	73.0	66.8	1194.6	553.5	542.9	4.9
(C) Waste Volume > 2500 ml										
95-02224	1/22/96	**2683**	18	537	72.2	63.3	603	882	560	4.47
	2/1/96	**2816**	18	352	69.6	65		420	455	4.24
8091485	2/6/96	**2626**	18	458	70.3	66.2		1158	543	4.86
373/85/1429	3/7/96	**2566**	32	689	74.3	71.9	1202	1049	515	5.18
373/85/1429	3/7/96	**2538**	34	678	73.9	70.1	1202	723	536	4.81
Average		**2645.8**	24.0	542.8	72.1	67.3	1002.3	846.4	521.8	4.7

Table 20: Effect of pressure on 214 12R6H Filter Performance w/ Frozen/Thawed Cells

Unit #	Date of Test	Max. Pressure	Rotor Speed	Nominal Flow Rate	Washing Time	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-Dilute Sub Hb	Post - wash - Sup Hb	Waste Sup. Hb.	Waste volume	Intra K+ mEg/ml
(A) Transmembrane pressure <300 mm Hg												
95-02217	1/4/96	**294**	800	100	13	61.9	44.8	862	935	1068	1638	
95-02218	1/11/96	**267**	800	150	12			408	719	566	1942	
8060243	2/1/96	**251**	800	150	25	69.8	66.7		882	663	2284	4.67
95-01585	2/8/96	**157**	800	150	15	75.5	65.8	697	678	410	2417	5.1
95--02205	2/12/96	**195**	800	150	11	68.2	64.3	284	606	630	1931	4.87
1628/1631a	2/22/96	**252**	600	100	20.0	73.9	69	2633	386	547	2279	4.66
Average		**236.0**	766.7	133.3	16.0	69.9	62.1	976.8	701.0	647.3	2081.8	4.8
(B) Transmembrane pressure 300 - 400 mm Hg												
95-02227	1/11/96	**352**	800	150	13			371	864	530	1963	
95-02230	1/22/96	**372**	800	150	15	73.7	66.4	641	652	504	2453	4.44
	2/1/96	**352**	800	150	18	69.6	65		420	455	2816	4.24
95-01634	2/8/96	**396**	800	150	15	66.3	50.8	1111	556	700	2373	5.51
1628/1631 b	2/22/96	**366**	800	100	20.0	73.5	73.9	560	220	555	2264	4.68
Average		**367.6**	800.0	140.0	16.2	70.8	64.0	670.8	542.4	548.8	2373.8	4.7
(C) Transmembrane pressure 401 - 500 mm Hg												
95-02220	1/4/96	**470**	800	150	13	76.9	70.5	618	1158	753	1801	
8091485	2/6/96	**458**	800	150	18	70.3	66.2		1158	543	2626	4.86
1583/1616b	3/4/96	**444**	800	100	30	71.8	70.3	1798	522	814	1924	5.07
379/89/1411	3/6/96	**435**	800	75	47	75.1	68.7	1518	424	470	2490	4.76
Average		**451.8**	800.0	118.8	27.0	73.5	68.9	1311.3	815.5	645.0	2210.3	4.9
(D) Transmembrane pressure > 500 mm Hg												
95-02224	1/22/96	**537**	800	150	18	72.2	63.3	603	882	560	2683	4.47
95-01568	2/12/96	**580**	800	150	20	69.2	71.5	729	731	784	1845	4.94
373/85/1429	3/7/96	**689**	800	50	32	74.3	71.9	1202	1049	515	2566	5.18
373/85/1429	3/7/96	**678**	800	50	34	73.9	70.1	1202	723	536	2538	4.81
Average		**621.0**	800.0	100.0	26.0	72.4	69.2	934.0	846.3	598.8	2408.0	4.9

Table 21: Effect of Filter Rotor Design on Performance of Washing Frozen-Thawed Blood

Unit #	Filter Type	Date of Test	Rotor Speed	Nominal Flow Rate	Washing Time	Max. Pressure	Post - WasteHb Recovery	Post - Pre/Post Hb Recovery	Pre-dilute Sup Hb	Post -wash - Sup Hb	Waste Sup Hb.	Intra K+ mEg/ml
Set (A)												
1583/1616b	214 12R-6H	3/4/96	800	100	30	444	71.8	70.3	1798	522	814	5.07
1583/1616a	214-12R	3/4/96	800	100	30	409	72.8	69.3	1798	642	763	4.37
Set (B)												
1379/89/1411a	214 12R-6H	3/6/96	800	75	47	435	75.1	68.7	1518	424	470	4.76
1379/89/1411b	214 6R-6H	3/7/96	800	100	38	602	73.6	76	1518	422	551	4.55
Set (C)												
1373/85/1429a	214 12R-6H	3/7/96	800	50	32	689	74.3	71.9	1202	1049	515	5.18
1373/85/1429b	214 12R-6H	3/7/96	800	50	34	678	73.9	70.1	1202	723	536	4.81
1373/85/1429c	214 6R-6H	3/7/96	800	60	28	769	75.9	73.3	1202	630	494	5.31

Table 22 - Modified Process and Disposable Configuration

Pool #	Description	Recovery using Waste hgb (%)	Recovery using Pre/Post hgb (%)	Post Sup hgb (mg/dl)	hgb Released (mg)
1	Unmodified	69	75	715	17,262
	Modified	88	86	345	5,841
2	Unmodified	49	52	1604	21,098
	Modified	71	78	393	14,018

Table 23 - Modified Process and Disposable Configuration (Average ± Standard Deviation)

	Unmodified	Modified	Modified with 3R 6H
Recovery using Waste hgb (%)	72 ± 4	76 ± 9	82 ± 5
Recovery using Pre/Post hgb (%)	66 ± 8	80 ± 6	82 ± 6
Post Sup hgb (mg/dl)	677 ± 252	625 ± 250	528 ± 179
hgb Released (mg)	14,276 ± 2,747	11,666 ± 5,319	7,910 ± 3,684
n	15	7	27

Table 24 - Rotor Design: 1.2μm vs 3.0μm Loprodyne (Average ± Standard Deviation)

	12 Ridge, 6 Hole	6 Ridge, 6 Hole	3 Ridge, 6 Hole
Recovery using Waste hgb (%)	73 ± 2	78 ± 1	79 ± 6
Recovery using Pre/Post hgb (%)	79 ± 4	80 ± 4	85 ± 5
Post Sup hgb (mg/dl)	672 ± 213	442 ± 106	395 ± 218
hgb Released (mg)	13,598 ± 1,998	9,773 ± 1,902	9,573 ± 4,189
n	4	3	4

Table 25 - Filter Media Evaluation (Average ± Standard Deviation)

	1.2μm	3.0μm
Recovery using Waste hgb (%)	85 ± 4	85 ± 3
Recovery using Pre/Post hgb (%)	85 ± 1	86 ± 3
Post Sup hgb (mg/dl)	534 ± 104	403 ± 116
hgb Released (mg)	6,528 ± 1,737	6,277 ± 842
Maximum Pressure (mm Hg)	193 ± 35	281 ± 60
n	4	4

Table 26 - Full and Partial T-106 Performance

Max Pressure (mmHg)		
Expt. #	*Partial T-106*	*Full T-106*
SF96009, 10	550	609
SF96013, 12	306	269
SF96015, 16	214	270
SF96023, 22	249	319
Mean	330	367
St. Dev.	152	163
p (two-tail t-test)	Not Significant	

Post Sup Hgb (mg/dL)		
Expt. #	*Partial T-106*	*Full T-106*
SF96009, 10	1092	1000
SF96013, 12	181	561
SF96015, 16	436	389
SF96023, 22	456	771
Mean	541	680
St. Dev.	388	264
'p (two-tail t-test)	Not Significant	

Table 27 - 3.0 μm Loprodyne and Biodyne A Performance

Max Pressure (mmHg)		
Expt. #	*3 LP*	*3 BA*
SF96034, 35	402	228
SF96039, 41	231	114
SF96046, 48	398	240
SF96055, 57	519	420
Mean	388	251
St. Dev.	118	126
p (one-tail t-test)	< 0.0022	

Post Sup Hgb (mg/dL)		
Expt. #	*3 LP*	*3 BA*
SF96034, 35	518	602
SF96039, 41	454	377
SF96046, 48	772	395
SF96055, 57	560	457
Mean	576	458
St. Dev.	138	102
p (two-tail t-test)	Not Significant	

Post Hct		
Expt. #	*3 LP*	*3 BA*
SF96034, 35	52	58
SF96039, 41	48	49
SF96046, 48	56	61
SF96055, 57	58	60
Mean	54	57
St. Dev.	4	5
p (one-tail t-test)	< 0.0302	

Table 28 - 1.2 mm Loprodyne and Biodyne A Performance

Max Pressure (mmHg)		
Expt. #	*1.2 BA*	*1.2 LP*
SF96036, 37	234	251
SF96052, 50	460	447
SF96056, 54	426	412
Mean	373	370
St. Dev.	122	105
p (two-tail t-test)	Not Significant	

Post Sup Hgb (mg/dL)		
Expt. #	*1.2 BA*	*1.2 LP*
SF96036, 37	642	419
SF96052, 50	1191	1197
SF96056, 54	510	717
Mean	781	778
St. Dev.	361	393
p (two-tail t-test)	Not Significant	

Post Hct		
Expt. #	*1.2 BA*	*1.2 LP*
SF96036, 37	54	57
SF96052, 50	58	59
SF96056, 54	60	60
Mean	57	59
St. Dev.	3	2
p (two-tail t-test)	Not Significant	

Table 29 - 3.0 μm Loprodyne and Supported 1.0 μm Cyclopore PC Performance

Max Pressure (mmHg)		
Expt. #	*3 LP*	*1 C PC*
SF96064, 65	391	157
SF96079, 77	503	299
SF96099, 100	677	660
SF96102, 103	1050	1360
SF96108, 109	675	684
Mean	659	632
St. Dev.	250	466
p (two-tail t-test)	Not Significant	

Post Sup Hgb (mg/dL)		
Expt. #	*3 LP*	*1 C PC*
SF96064, 65	616	551
SF96079, 77	535	583
SF96099, 100	509	398
SF96102, 103	524	498
SF96108, 109	527	408
Mean	542	488
St. Dev.	42	83
p (two-tail t-test)	Not Significant	

Post Hct		
Expt. #	*3 LP*	*1 C PC*
SF96064, 65	45	54
SF96079, 77	57	61
SF96099, 100	50	48
SF96102, 103	48	43
SF96108, 109	54	51
Mean	51	51
St. Dev.	5	7
p (two-tail t-test)	Not Significant	

Table 30 - 3.0 μm Loprodyne and Supported 1.0 μm Cyclopore PET Performance

Max Pressure (mmHg)		
Expt. #	*3 LP*	*1 C PET*
SF96064, 66	391	90
SF96071, 70	1220	322
SF96075, 73	805	345
SF96079, 76	503	273
SF96099, 101	677	760
SF96102, 104	1050	1014
SF96105, 107	884	895
Mean	790	528
St. Dev.	293	355
p (two-tail t-test)	Not Significant	

Post Hct		
Expt. #	*3 LP*	*1 C PET*
SF96064, 66	45	55
SF96071, 70	45	66
SF96075, 73	53	66
SF96079, 76	57	66
SF96099, 101	50	47
SF96102, 104	48	46
SF96105, 107	46	44
Mean	49	56
St. Dev.	5	10
p (two-tail t-test)	Not Significant	

Table 31 - 3.0 μm Loprodyne and Unsupported 1.0 μm Cyclopore PET Performance

Post Sup Hgb (mg/dL)		
Expt. #	*3 LP*	*PET us*
SF96112, 113	753	567
SF96120, 121	408	339
SF96122, 123	532	375
SF96124, 125	587	475
Mean	570	439
St. Dev.	143	103
p (one-tail t-test)	< 0.0073	

Max Pressure (mmHg)		
Expt. #	*3 LP*	*PET us*
SF96112, 113	1386	329
SF96120, 121	667	276
SF96122, 123	748	254
SF96124, 125	803	295
Mean	901	289
St. Dev.	328	32
p (one-tail t-test)	< 0.0134	

Post Hct		
Expt. #	*3 LP*	*PET us*
SF96112, 113	42	68
SF96120, 121	51	66
SF96122, 123	50	65
SF96124, 125	48	65
Mean	48	66
St. Dev.	4	1
p (one-tail t-test)	< 0.0028	

Waste Recovery (%)		
Expt. #	*3 LP*	*PET us*
SF96112, 113	81	87
SF96120, 121	88	93
SF96122, 123	90	93
SF96124, 125	86	92
Mean	86	91
St. Dev.	4	3
p (one-tail t-test)	< 0.0026	

Hgb Released (mg)		
Expt. #	*3 LP*	*PET us*
SF96112, 113	8706	5336
SF96120, 121	5228	2850
SF96122, 123	4660	3186
SF96124, 125	6448	3453
Mean	6261	3706
St. Dev.	1793	1114
p (one-tail t-test)	< 0.0043	

Hgb Retained (mg)		
Expt. #	*3 LP*	*PET us*
SF96112, 113	1512	426
SF96120, 121	579	277
SF96122, 123	789	307
SF96124, 125	919	403
Mean	950	353
St. Dev.	400	72
p (one-tail t-test)	< 0.0196	

Table 32: Effect of Rotor Speed on TBPS Performance with Plain Rotors

Post Hematocrit				
RPM	*1200*	*1400*	*1600*	*1800*
Average	65.2	67.8	68.3	62.7
Std. Dev.	2.1	1.0	1.3	6.0
n	6.0	4.0	4.0	3.0
P-Value vs 1400	0.034	-	N.S.	N.S.

Post Supernatant Hb				
RPM	*1200*	*1400*	*1600*	*1800*
Average	416.5	342.3	341.0	248.0
Std. Dev.	145.1	52.9	105.8	80.7
n	6.0	4.0	4.0	3.0
P-Value vs 1400	N.S.	-	N.S.	N.S.

Waste Hb Recovery (%)				
RPM	*1200*	*1400*	*1600*	*1800*
Average	85.9	86.3	84.4	74.0
Std. Dev.	1.9	1.6	1.3	7.1
n	6.0	4.0	4.0	3.0
P-Value vs 1400	N.S.	-	N.S.	N.S.

Pre/Post Hb Recovery (%)				
RPM	*1200*	*1400*	*1600*	*1800*
Average	83.8	81.7	80.9	72.1
Std. Dev.	1.6	3.3	2.5	6.5
n	6.0	4.0	4.0	3.0
P-Value vs 1400	N.S.	-	N.S.	N.S.

Table 33: Effect of Rotor Speed on Peformance of TBPS with 3R Rotor

Rotor Speed (RPM)	Post Hematocrit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
800	65	488	85	81
800	68	795	87	84
800	63	752	87	87
Average	**65.3**	**678.3**	**86.2**	**84.1**
1000	69	517	84	81
1000	72	929	87	81
1000	71	402	87	85
Average	**70.7**	**616.0**	**86.2**	**82.2**

Table 34: Effect of Magnet Well Seal on Performance of TBPS.

Seal		Post Hematocrit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)
control	average	67	338.5	86.6
	Std. Dev.	0.8	108.1	2.1
0.005	average	64.3	444.3	82
	Std. Dev	1	109.7	2.8
	p-value vs. control	0.005	N.S.	0.042
0.01	average	64	491.3	82.1
	Std. Dev	1.4	152.6	2.8
	p-value vs. control	0.01	N.S.	0.045

Table 35: Effect of Post Thaw Diluent on TBPS Performance

Diluent Sequence Post Thaw		Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Waste Sup. hgb (mg/dl)
12/0.9	Average	330.4	85.17	377.9
	StDev	88.4	3.27	83.4
8.5/1.6	Average	307.9	78.6	548.4
	StDev	63.72	4.25	113.1
	P value	N.S.	0.004	0.005

Table 36: Effect of Warming Saline on the Performance of TBPS

Parameter	Room Temperature	Warmed Saline
Post hematocrit	65.0	68.0
Post supernatant hgb.	374.5	303.3
Waste hgb recovery	82.5	85.4
pre/post hgb recovery	81.1	84.0

Table 37: Effect of In-Line Mixing and Sealing Disposable on TBPS Performance

Goal of Test	Test #	Post Hematocrit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
control	SF96217	70	318	91	90
mixing	SF96218	70	298	90	89
mix+clmp	SF96219	68	**237**	92	87
control	SF96220	69	380	88	89
clamped	SF96221	69	245	89	93
mix+clmp	SF96222	68	**146**	88	89

Table 38: Performance of Current Configuration of TBPS Process with Individual Units

Test #	Date of Test	Max. Pressure (mmHg)	Volume of RBCs In (ml)	Pre Sup. hgb (mg/dl)	Post Hemato crit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre/ Post hgb (%)	Post Thaw Sup. Hb Adjusted Waste Hgb Recovery (%)
SF96224	12/19/96	194	128	1103	66	171	80	78	88.30
SF96225	12/19/96	352	190	6524	63	181	82	81	93.04
SF96226	12/19/96	81	177	919	71	274	94	92	95.06
SF96227	12/20/96	-18	128	5006	54	165	86	87	89.57
SF96228	12/20/96	69	189	3089	69	355	85	88	89.14
SF96229	12/23/96	-6	158	2054	67	261	92	90	93.69
SF96230	12/23/96	330	205	7289	68	124	77	77	89.27
Average			167.8	3712.0	65.4	218.7	85.2	84.7	91.2
Std. Dev.			30.8	2585.9	5.6	80.6	6.2	6.2	2.7

Table 39: Results for Individual Units Washed w/ Automated TBPS

Test #	Pre Sup. Hgb	Post Sup. Hgb	Post-Thaw Recovery	Post-Wash Pre/Post Hgb Recovery	Post-Wash Waste Hgb Revovery	Post Wash Hematocrit
A. Pre-Wash Supernatant Hgb < 5000 mg/dL						
057	78	551	99.8	77	87.5	70
058	113	284	99.9	73.4	83.1	66
053	272	102	99.5	88.9	95.54	60
024	1299	199	96.8	100.6	66.62	57
032	1394	336	97.9	82.8	88.4	69
047	1483	218	98	45.1	85.2	65
023	1846	236	97.4	83.4	87.2	67
037	3703	205	92.7	72.8	77.7	61
036	3778	357	96.32	77	80.8	60
017	4622	206	94.3	87.1	85.3	70
Average	1858.8	269.4	97.3	78.8	83.7	64.5
Stdev	1637.3	123.2	2.4	14.5	7.7	4.7
B. Pre-Wash Supernatant Hgb > 5000 mg/dL						
026	5367	443	91.2	73.8	78.4	65
022	5472	599	94.7	73.7	75.9	65
025	6193	217	91.5	72.3	78.8	
031	6349	142	88.6	68.2	78	60
056	18658	371	82.8	70.3	73	
055	18738	170	84.2	84.4	92.5	62
063	19201	387	87.5	71.6	76.9	61
061	20776	351	89	72.3	77	65
035	21349	683	83.3	69.3	73.8	60
062	23458	512	83	72.7	75.7	70
060	25263	471	84.8	75.5	78.3	67
045	28322	298	82.6	68.6	73.4	55
Average	16595.5	387.0	86.9	72.7	77.6	63.0
Stdev	8412.4	166.2	4.1	4.3	5.1	4.3

Table 40: Performance of 320 12R6H Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash Sup. Hb (mg/dL)
116	20.0	100	900	178	63.8	60.8	95.3		
130	18.5	100	900	88	64.7	60.8	93.9		
124	36.0	150			64.9	53.1	81.8		
136	14.0	100	800	111	65.5	61.4	93.7	320	76
137	12.0	150	800	145	65.5	61.2	93.4	320	885

Table 41: Performance of 518 Plain (WUPI) Filter for Washing Fresh Blood Cells

Test #	Total Wash Time (min)	Set Flow Rate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Pre-Wash PHb (mg/dL)	Post-Wash PHb (mg/dL)
32	21.4	80	1200	153	43.1	42.3	98.2	33	216
33	21.0	150	1200	124	44.9	45.9	102.3	42	270
31	23.8	80	1200	280	51.7	52.7	101.9	33	244
35	16.5	50	1200	380	56.6	50.1	88.5	49	471

Table 42: Comparison of Results for Washing Two Units in Series with Each Filter Design

Test #	Filter Type	Back Flushed	Dilute Blood Volume In (mL)	Saline Wash Volume (mL)	Total Wash Time (min)	Average Flowrate (mL/min)	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Target Hct.	Post Hct	Hct. Recovery	Calcium Washout (%)
44	214 pln	no	700	1000	16.5	103	1200	92	59.9	61.7	103.1	99.31
45	214 pln		700	1000	16.5	103	1200	295	59.9	51.8	86.5	99.28
60	214 pln	no	878	1000	17.5	107	1200	356	76.7	64.5	84.1	98.57
61	214 pln		882	1000	26.0	72	1200	521	77.1	60.5	78.5	99.28
114	214 12R 6H	no	735	1000	18.0	96	900	153	68.0	63.2	92.9	
115	214 12R 6H		733	1000	19.0	91	900	339	67.8	48.7	71.8	
128	214 12R 6H	yes	602	1000	15.0	107	900	117	64.7	62.5	96.6	99.52
129	214 12R 6H		601	1000	15.3	105	900	133	64.6	60.5	93.6	99.50
130	320 12R 6H	yes	903	1000	18.5	103	900	88	64.7	60.8	93.9	99.50
131	320 12R 6H		901	1000	17.8	107	900	113	64.6	57.5	89.0	99.24

Table 43: Results of Washing Two Units in Series w/ Final Filter Configuration

	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Test #	Post Sup. Hgb	Post Sup. Hgb	Post-Wash Pre/Post Hgb Recovery	Post-Wash Pre/Post Hgb Recovery	Post-Wash Waste Hgb Revovery	Post Wash Waste Hgb Recovery	Post Wash Hematocrit	Post Wash Hematocrit	Max Pressure	Max Pressure
03/04	281	283	81.9	83.8	81.7	81.4	66	62	118	143
05/06	232	243	81.1	84.3	81.4	83.3	65	60	176	315
07/08	339	298	87.3	89	87.6	87.3	68	68	177	275
09/10	314	415	84.5	86.8	86	86.2	69	68	148	217
11/12	270	174	70.4	68	68.1	66.5	60	60	86	94

Table 44: Results for Washing Thawed Frozen Blood in a Flexible Volume Recirculating Circuit

												Goal >80%	Goal >80%	Goal <150	Goal >=70
Notes	Goal of Test	Test #	Date of Test	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Weight of Blood In (g)	Pre Sup. hgb (mg/dl)	Pre Total Sup hgb / unit (mg)	Waste Sup. Hb. (mg/dl)	Post Osmo (mOs/kg H_2O)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post - Hematocrit
Did not put in enough blood to concentrate	To determine if circuit can concentrate and washout glycerol	B	7/2/96	600	72	779	510		324	1223			825	58
Did not put in enough blood to concentrate	To determine if circuit can concentrate and washout glycerol	C	7/3/96	800	80	311	1091		168	645			833	56
Did not put in enough blood to concentrate	To determine if circuit can concentrate and washout glycerol	D	7/5/96	900	82	513	844	2041	335	820	80.8	60.9	1498	66.1
Used enough blood to concentrate	To determine loads at high osmo and hct for recirc. circuit	E	7/8/96	900	87	627	1846	4272	464	1223	83.4	60.3	1832	78
Did not run long enough to concentrate	To determine loads at high osmo and hct for recirc. circuit	F	7/9/96	900	73	643	600	1354	563	1470	76.7	51.1	1709	64
Used enough blood to concentrate	To determine loads at high osmo and hct for recirc. circuit	G	7/9/96	900	72	620	600	1305	541	1563	75.1	45.9	2632	73
Accomplished pumping	To determine if pumping the recirc. and holding at 100 mmHg helps washout	SF96001	7/11/96	800	155	687	1074	2723	557	1171	85.3	80.6	1897	75
Accomplished pumping	Repeat of SF96001 except use Full T-106 bonded to ribs	SF96002	7/12/96	800	160	729	1407	4349	472	1130	84	72.7	1683	69
Accomplished pumping and pressure	Repeat of SF96002 except pressure goes to 150 mmHg	SF96003	7/12/96	800	162	716	1407	4271.7	495	1192	83.9	70.2	1860	70
Run too long - hgb in filter went up rapidly at end of run	Use more saline to see if glycerol washes out better for large units	SF96004	7/15/96	800	146	603	704	1566	199	1099	87.8	79	2356	57
hematocrit in filter not high enough	Use higher hct during recirc/wash to improve glycerol washout	SF96005	7/16/96	800	399	673	568	1515	464	1078	86.3	72.1	1512	74
hematocrit not controlled well enough	Use dil./conc. during wash to improve glycerol washout	SF96006	7/16/96	800	380	571	568	1286	304	913	90.1	78.7	932	70
hematocrit in filter not high enough	Use higher hct during recirc/wash to improve glycerol washout	SF96007	7/18/96	800	445	666	560	1275	722	1254	80.3	70	2023	73
hematocrit not controlled well enough	Use dil./conc. during wash to improve glycerol washout	SF96008	7/18/96	800	566	660	560	1263	659	965	82.8	74.9	1143	58

Table 45: Performance of TBPS Washing Rejuvenated and Non-Rejuvenated Frozen Blood

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
Non-Rejuvenated Blood															
SF96082	8/22/96	3R6H	3 LP	24	520	175	318	1221	286	2080	5400	87	83	481	50
SF96083	8/22/96	3R6H	3 LP	24	476	175	318	1165	303	2088	5850	86	83	507	50
SF96084	8/22/96	3R6H	3 LP	24	438	175	318	1884	255	2097	3995	88	86	441	54
SF96094	8/27/96	3R6H	3 LP	24	581	175	338	666	280	2091	5907	87	87	539	53
SF96095	8/27/96	3R6H	3 LP	24	461	181	338	578	216	2113	4526	90	92	449	56
SF96096	8/27/96	3R6H	3 LP	24	509	178	338	608	241	2095	6149	87	86	1276	52
Rejuvenated Blood															
SF96085	8/23/96	3R6H	3 LP	23	535	164	255	766	290	2011	6041	86	82	665	48
SF96086	8/23/96	3R6H	3 LP	23	520	164	255	728	247	2012	4904	88	80	491	51
SF96087	8/23/96	3R6H	3 LP	23	514	164	255	801	240	2014	4646	89	83	468	52
SF96091	8/26/96	3R6H	3 LP	24	510	175	277	954	219	2016	3936	91	90	358	54
SF96092	8/26/96	3R6H	3 LP	24	700	178	277	947	263	2024	5041	89	91	466	52
SF96093	8/26/96	3R6H	3 LP	24	718	180	277	1052	267	2016	5007	88	89	458	51

All Operating Paramteres are 800 rpm, 100 ml./min, with 3R 6H rotors

Table 46: Summary of Historical Progress in Recovery and Post Supernatant Hemoglobin

		Recovery *			Post Sup. Hg		
Time Period	**Processing Description**	**Average**	**n**	**P-value vs. current**	**Average**	**n**	**P-value vs. current**
Current w/ Auto Prototype	PET 1u Plain rotor with reverse drip chamber mixing and sealed disposable	**83.7**	**10**		**269.4**	**10**	
Current w/ Manual Prototype	PET 1u Plain rotor with reverse drip chamber mixing and sealed disposable	**86.2**	**9**		**212.7**	**9**	-
End of Phase I Report	LP 1.2u Plain Rotor Disposable	73.4	12	0.0004	511.8	12	0.0005
End Year 1	LP 1.2u 12R Rotor Disposable	71.9	20	0.00003	692.5	20	7.1E-09
Fifth Quarter	LP 1.2u 12R Rotor Disposable	71.9	15	0.00002	677.5	15	3.10E-06
Fifth Quarter	Modified Prime, Mixing, and Flow Path LP 12R 1.2u Disposable	76.4	7	0.035	625.3	7	0.004
Fifth Quarter	Modified Prime, Mixing, and Flow Path LP 3R 1.2u Disposable	81.7	27	N.S.	527.7	27	1.90E-08
Sixth Quarter	Modified Prime, Mixing, and Flow Path LP 3R 3.0u Disposable	86.3	4	N.S.	570	4	0.01
Six Quarter	Modified Prime, Mixing, and Flow Path PET 1u unsupported media with 3R rotor Disposable	92.3	4	N.S.	439	4	0.013

*Recovery calculated from waste hemoglobin level.

Sec.	TABLE 47 Disposable Feature	Completed	Description of Feature/Component
1	**DESCRIPTION/INTENDED USE**	Y/N	
	Deglycerolization of thawed frozen blood utilizing a closed loop, sterile system. This specification covers the filter disposable portion of the system described in the previous sections of the proposal. Refer to system specifications for operating requirements.	Y	See Appendix D for details.
2	**REGULATORY STATUS**		
	The device will meet or exceed all applicable FDA licensing and manufacturing requirements. The system disposables are considered accessories to the TBPS system and are regulated as such.	Y	
3	**PERFORMANCE REQUIREMENTS**		
	The device will be capable of washing one and/or two units of thawed cells with one disposable filter cartridge.	Y	Feasibility of 2 units washed in series demonstrated w/comparable results for each (Table 43)
	The device must accommodate the addition of an additive nutrient to the washed cells for prolonged shelf life survival.	N	During course of development, additive solutions were mutually a low priority of Contract Officer & AHT. Decision to have this evaluated by Dr. Meryman.
	Recovery and survivability of washed cells will at a minimum, meet the requirements as stated in FM8-70, the Standards for Blood Banks and Transfusion Services of the American Association of Blood Banks.	Y/N	Recovery is acceptable immediately post-wash. Survival was not evaluated in Baboons or Man.
	Acceptable range of final hematocrit after adding additives is 50-60%.	N	Not at this time, however, feasibility of a recirculating system which can give HCT up to 75% has been demonstrated and shall meet this objective (Table 44)
	85% or more of the RBC's should be recovered in the deglycerolizing process.	Y/N	Supernatant hgb levels average at best ≈ 200 mg/dL. All other criteria met.
	The end blood product will have the following characteristics: residual glycerol less than 1%, initial supernatant Hb levels of 150 mg/dL or less, absence of significant potassium leaks.	N	
	The device must operate without degradation in temperature from 10 to 37 degrees C. The system will survive storage without degradation in temperatures from -40 to 70 degrees C.	N	
	Wash time of less than 20 minutes.	Y	
4	**PHYSICAL REQUIREMENTS**		
	The device will be a closed sterile system using sterile docking techniques to connect tubing to units of blood.	Y	
	0.22 micron filters will be used for accomplishing solution connections, venting and pressure monitoring.	Y	Figure 16
	A sterile docking device will be used for accomplishing connections to blood units.	Y	Figure 16
	To reduce risk of operator error, tubing will be color coded based on the Haemonetics 115	Y	All tubing and pinch valves are color coded

	TABLE 47		
	Tubing will accommodate flow metering devices for diluent solutions and wash solution.	N/A	Pump used for all solution flow.
	Tubing will accommodate directional valves for automatic fluid management into and out of the device, solution bags and blood bags.	Y	
	The outlet waste line tubing will incorporate one or more flow through cells for monitoring hemolysis and potentially monitoring glycerol concentration in the waste solution.	Y	Hemoglobin & Glycerol sensor installed.
	The outlet blood line tubing will incorporate a flow through cell for monitoring hematocrit of washed blood.	N	Not deemed a critical feature since all blood is contained in filter until after washing complete.
5	**BIOLOGICAL REQUIREMENTS**		
	Materials in the fluid path will be FDA approved class VI toxicity at 120 degrees C.	Y	
	Sterile product will be ETO sterilized and meet 1 x 10-6 sterility assurance.	N	Filter disposable is similar in size composition and packaging to a disposable which has had sterilization validation performed. Therefore any future sterilization validation should be able to utilize, in part such data.
	ETO residuals for sterilized product will be less than or equal to 20 ppm.	N	See above
6	**PACKAGING/LABELING REQUIREMENTS**		
	Each device will be packaged in a container that meets NSTA Project 1A (UPS) shiptest and MIL.STD-167-1 VIBRAT Conf. test with appropriate warnings and identification.	Y	
	Each individual container and master container will be labeled		

	TABLE 48		
	HARDWARE - CONSOLE		
1	**GENERAL DESCRIPTION**		
	This specification covers the hardware portion of the system described in the previous sections of the proposal. Refer to system specifications for operating requirements.		
2	**REGULATORY REQUIREMENTS**		
	The TBPS is described in ZICFR864.9145 Processing System for Frozen Blood. The device is classified as Class II within the hematology and pathology subpart J group of products used in establishments that manufacture blood and blood products.		
3.0	**PERFORMANCE REQUIREMENTS**		
3.1	**Control Requirements**		
	The device main power will be controlled via a double throw main power switch which disconnects both power lines.	Y	
	The device will incorporate a sealed membrane keypad for single button control of primary operating functions, including but not limited to: a. START processing, b. STOP processing, C> RESUME processing	Y	See Appendix D
3.2	**Data Storage Requirements**		
	The device will contain non-volatile memory so that parameters are retained during power outages and when the device is turned off.	Y	All data are stored in RAM
	The device will retain the following parameters:		
	a. Unit number of the thawed frozen red cell unit	Y	See software validation
	b. Unit number of the washed RBC unit	Y	See software validation
	c. Unit number of the sister unit	Y	See software validation
	d. Lot number of the 12% saline	Y	See software validation
	e. Lot number of the .9% saline	Y	See software validation
	f. Name and lot number of shelf life extension additive	N/A	
	g. Serial number of TBPS unit	Y	See software validation
	h. Expiration date of deglycerolized blood	Y	See software validation
	I. Start and stop time of wash process	Y	See software validation
	j. Hematocrit	N	
	k. Hemolysis estimate	Y	See software validation
	l. Glycerol concentration	Y	See software validation
3.3	**Display Requirements**		
	The display will provide for at least 80 alphanumeric characters in a 4 line by 20 character format or more.	Y	
	The display will be of the vacuum fluorescent type.	Y	

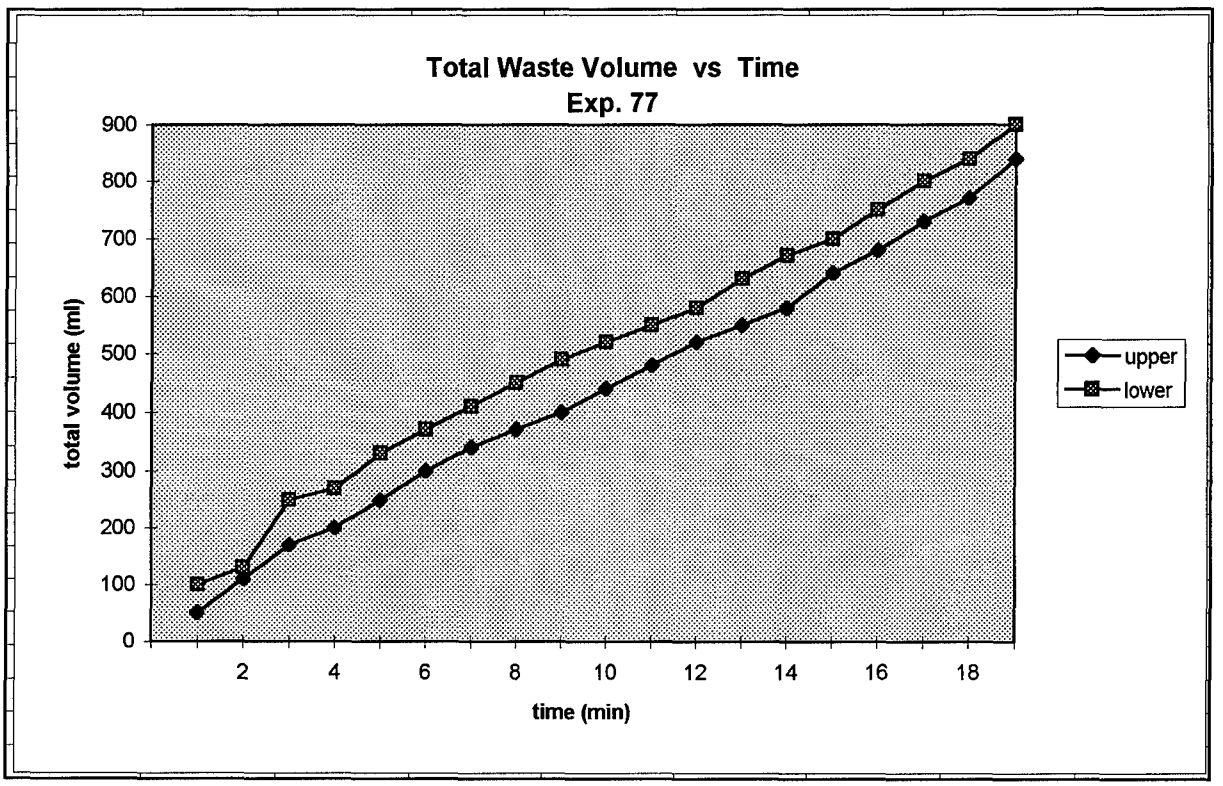
3.4	**Data Entry Requirements**		
	The device will allow data entry from either a bar code reader or a keyboard.	Y	
	The device will allow a current data value to be used in lieu of a new entry.	Y	
	The device will provide for validating data entries utilizing a checksum, double entry, or equivalent method.	Y	
	Keyboard Requirements:		
	The keyboard will be of the sealed membrane type.	Y	
	The keyboard will have a Key for each character in the full alphanumeric character set.	Y	
	Bar Code Reader Requirements:		
	The bar code reader will read CODABAR format.	Y	
	The bar code reader will read Code 128 format.	Y	
	The bar code reader will be a hand-held wand.	Y	
3.5	**Data Reporting Requirements**		
	The device will include a printer for recording and reporting parameter and performance data.	Y	Stand alone attached printer
	Each processed unit will generate a printed report including:		
	a. Unit number of the thawed frozen red cell unit	Y	
	b. Unit number of the washed RBC unit	Y	
	c. Unit number of the sister unit	Y	
	d. Lot number of the 12% saline	Y	
	e. Lot number of the .9% saline	Y	
	f. Name and lot number of shelf life extension additive	Y	
	g. Serial number of TBPS unit	Y	
	h. Expiration date of deglycerolized blood	Y	
	I. Start and stop time of wash process	Y	
	j. Hematocrit	N	
	k. Hemolysis	Y	
	l. Glycerol	Y	
	Printer requirements		
	The printer will be contained within the device console.	N	Stand alone attached printer
	The printer will support the full alphanumeric character set.		
3.6	**Audible Signal Requirements**		
	The device will contain an audible alert for notifying the operator of abnormal conditions.	Y	See software section
	An audible alert will occur if the device diagnostics detect a device malfunction.	Y	See software section
	An audible alert will occur if the performance monitors determine a product quality deviation.	Y	See software section

3.7	**Performance Monitoring Requirements**		
	The device will provide for measuring output hematocrit.	N	
	The device will provide for measuring hemolysis.	Y	
	The device will provide for measuring residual glycerol.	Y	
	The device will monitor in-line pressure and alarm for system over pressurization.	Y	
3.8	**Diagnostic Requirements**		
	The device will include the following start-up diagnostics:	Y	
	The device will perform a RAM memory test	Y	
	The device will perform a ROM checksum test	Y	
	The device will perform a CPU processing test	Y	
	The device will validate non-volatile parameters.	Y	
	Device power control tests will:		
	a. verify that the shutdown mechanism works	Y	See software validation report
	b. verify that the motor controller "off" control is operational	Y	Same as above
	Watchdog timer tests will include:		
	a. Determine power-up vs. Watchdog timer restart	N/A	
	b. Verify watchdog time-out and restart status	N/A	
	c. Start watchdog timer	N/A	
	d. Verify watchdog timer status	N/A	
	The device will test for valid analog readings.		
	The device will include the following run-time diagnostics:		
	Motors diagnostics will:		
	a. Verify motors are stopped when commanded off.	Y	See software validation report
	b. Verify motors run when commanded to do so.	Y	Same as above
	c. Verify that motor speed is within 5% of commanded speed.	Y	Same as above
	d. Detect motor over-load.	Y	Same as above
	The device will verify that analog values are within valid bounds	Y	
	The device will detect power failures.	N	
3.9	**Electrical Requirements**		
	The device power mains will be selectable between 110 and 220 VAC, +-15%.	Y	110 vac only . Phase 3 will include 220 vac
	The device operating frequency will be 50/60Hz, single phase.	Y	
	The device will meet FCC Class A regulations including EMI.	Y	
	The device will withstand 200% power surges of 100 microseconds or less without damage, data loss, or operational interruption.	Y	
	The device will withstand momentary power losses of 50 microsecond duration without damage, data loss, or operational interruption.	Y	
	The device power consumption will not exceed 600 watts.	Y	
	The device power cord will be NEMA 5-15R, hospital grade, and 10 foot in length.	Y	

3.10	**Environmental Requirements**		
	Device operating temperature range will be not less than 10-37 degrees Celsius	Y	
	Device storage temperature range will be not less than -40 to 70 °C.	Y	
	Device operating humidity range will meet ASHRAE Data Processing Space standards.	Y	
	The device will meet MIL-STD-167-1 vibration standards.	Y	
3.11	**Reliability Requirements**		
	Operational Mission MTBF will be 320 hours or greater.	Y	
	Unit Mean Time to Repair (MTTR) will be 1 hour or less.	Y	
	General MTTR will be 3 hours or less.	Y	
3.12	**Safety Requirements**		
	The device will monitor the microprocessor for proper functioning.	Y	See software validation report
	The device will stop all motive devices in the event microprocessor failure is detected.	Y	Same as above
4.0	**PHYSICAL REQUIREMENTS**		
4.1	**Dimensional Requirements**		
	12" wide x 24" length x 39" tall	Y	
4.2	**Weight**		
	110 lbs. maximum	Y	
4.3	**Materials of Construction**		
	Suitable to withstand the environmental requirements.	Y	
5.0	**PACKAGING AND LABELING**		
5.1	**Warnings and Precautions**		
	All warnings and precautions will be prominently displayed on the operating unit.	N	
5.2	**Packaging**		
	The packaged unit will withstand the MIL-STD-167-1 vibration test.	Y	
	The packaged unit will withstand NSTA Project 1A (UPS) Ship Test.	Y	

List of Figures

Figure 1: BUP Evaluation of Waste Volume Measurement, Upper and Lower



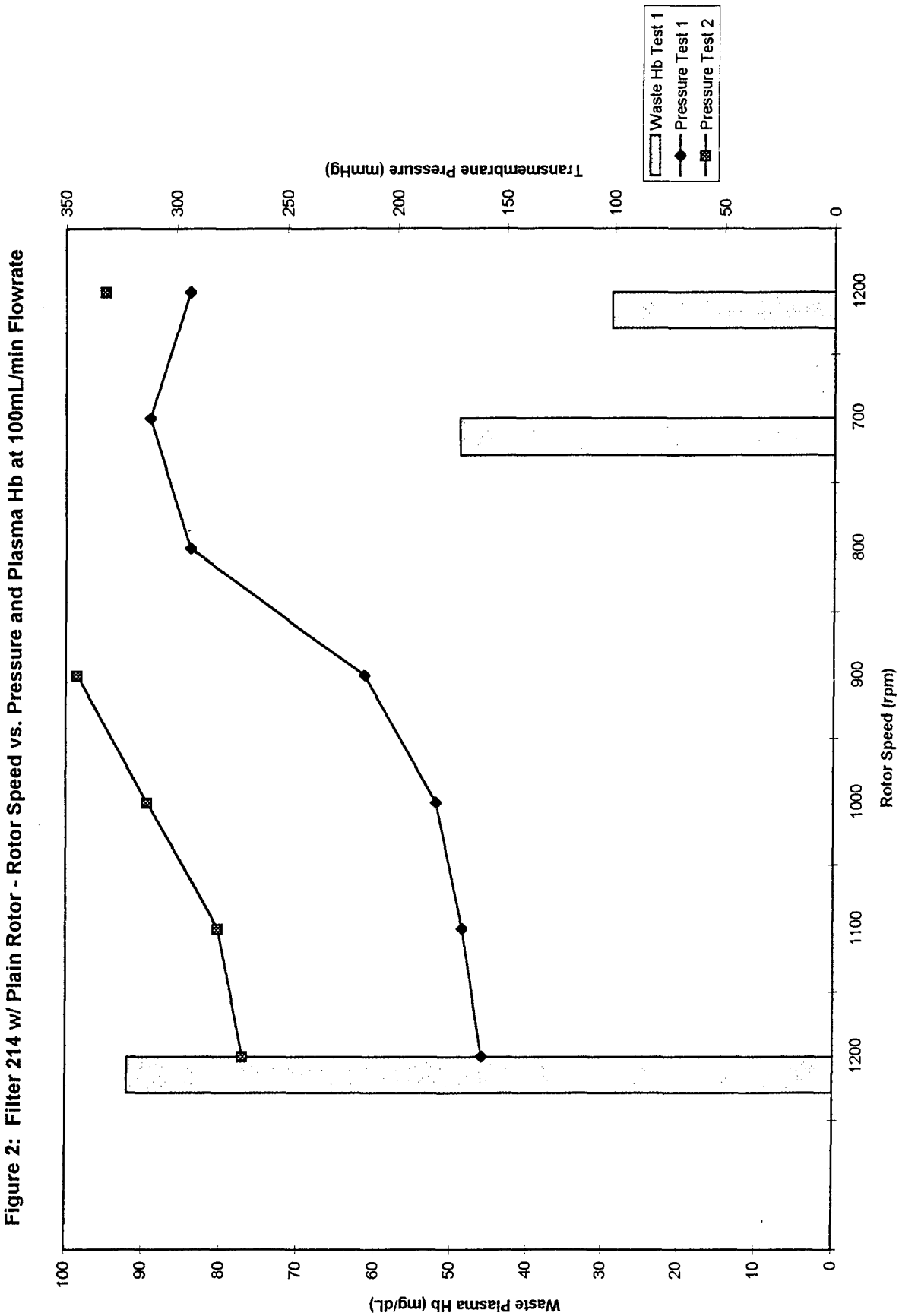
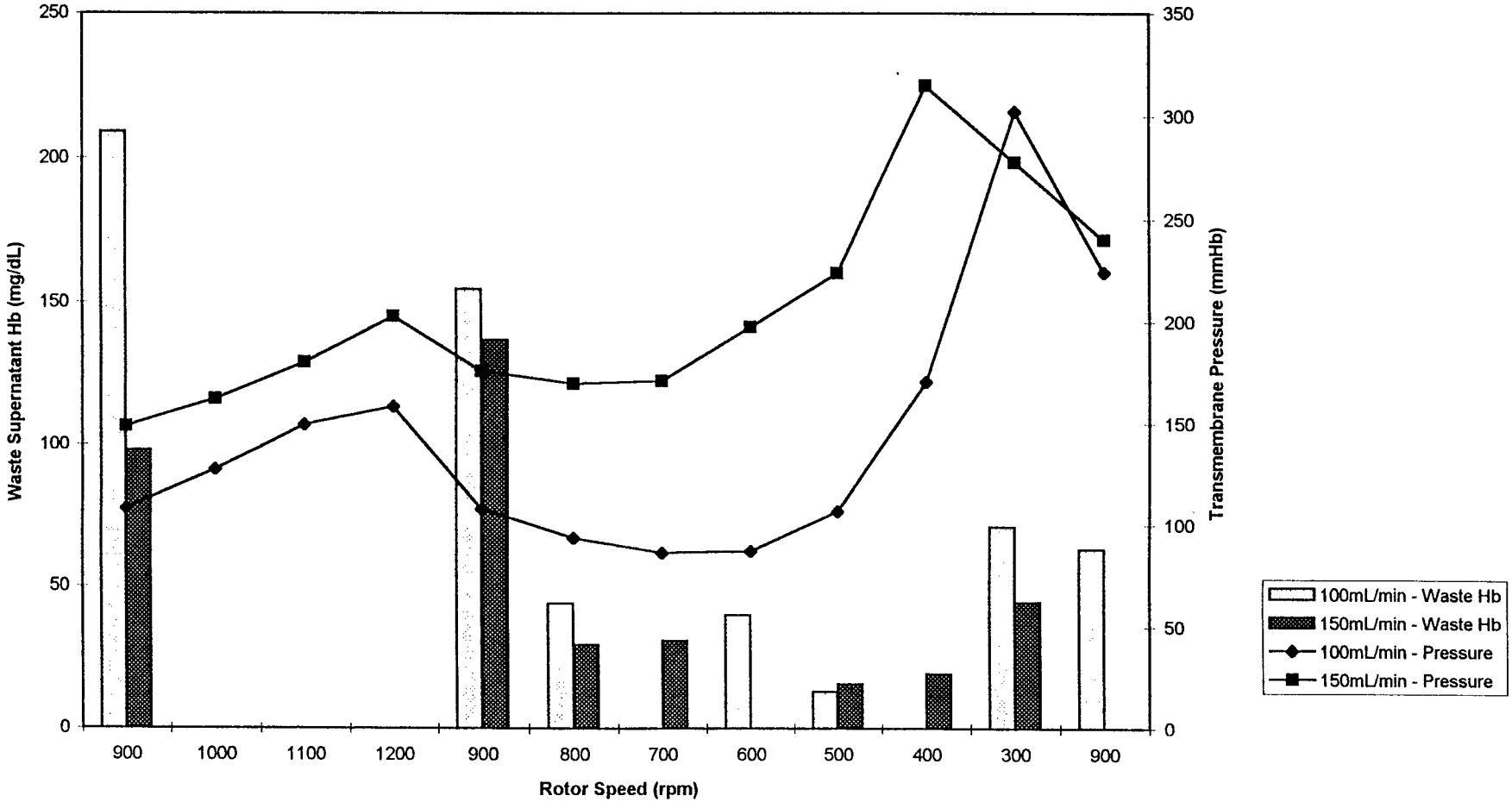


Figure 2: Filter 214 w/ Plain Rotor - Rotor Speed vs. Pressure and Plasma Hb at 100mL/min Flowrate

Figure 3: Filter 214 w/ 12R 6H Rotor - Comparison of Averaged Data for Rotor Speed vs Plasma Hb at 100mL/min vs 150mL/min Flowrate



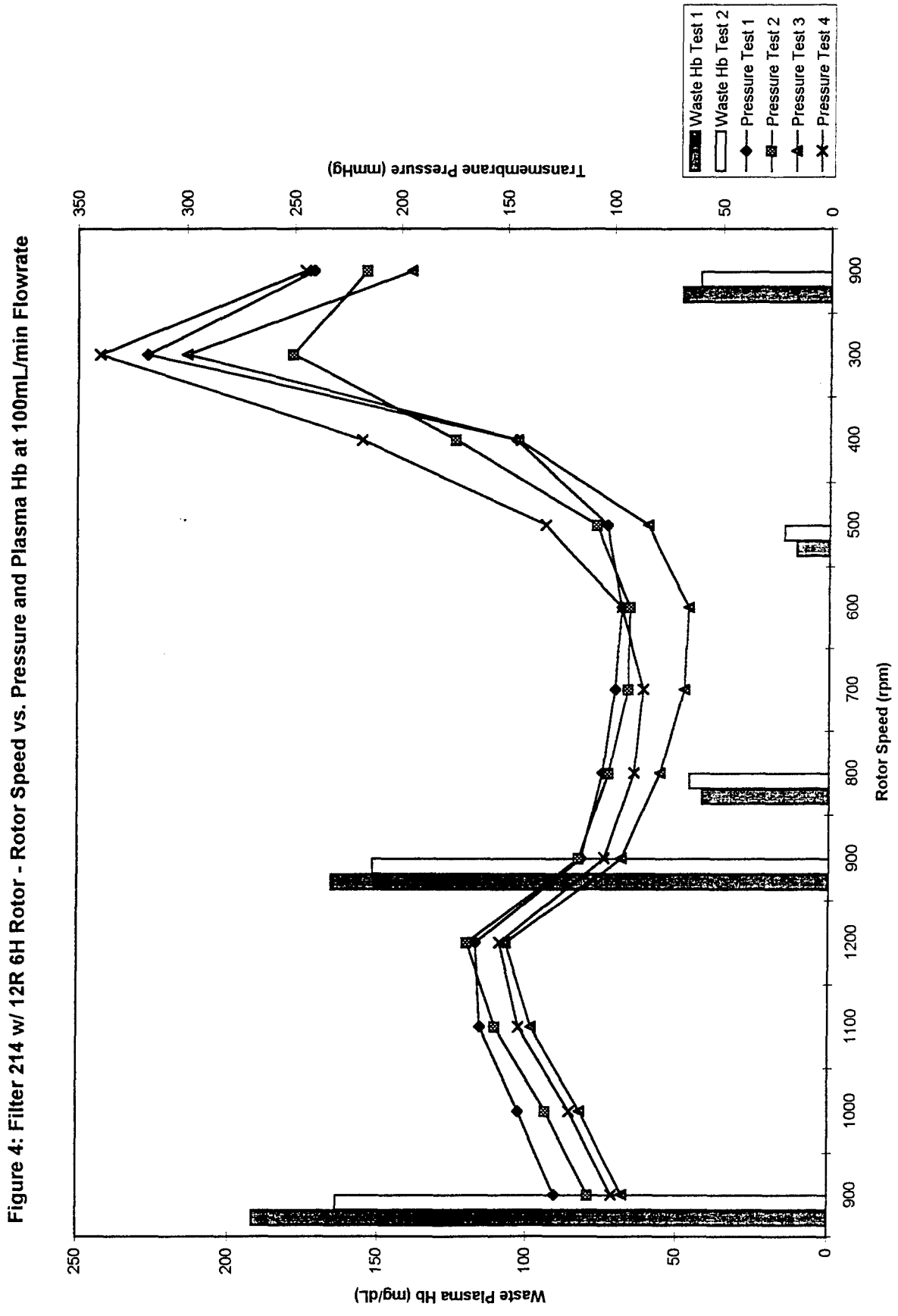


Figure 4: Filter 214 w/ 12R 6H Rotor - Rotor Speed vs. Pressure and Plasma Hb at 100mL/min Flowrate

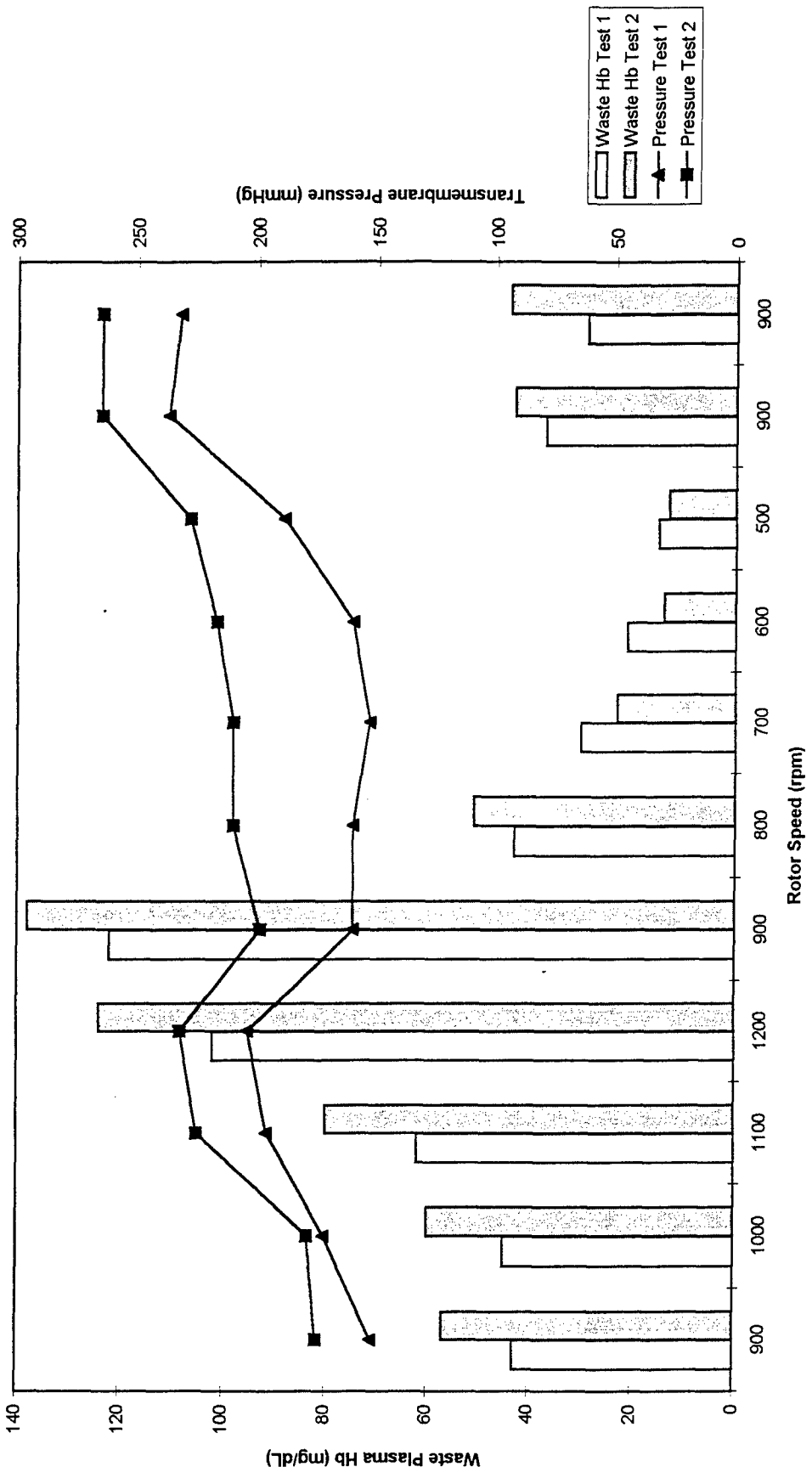


Figure 5: Filter 214 w/ 12R 6H Rotor - Rotor Speed vs Plasma Hb at 150mL/min Following Equilibrium

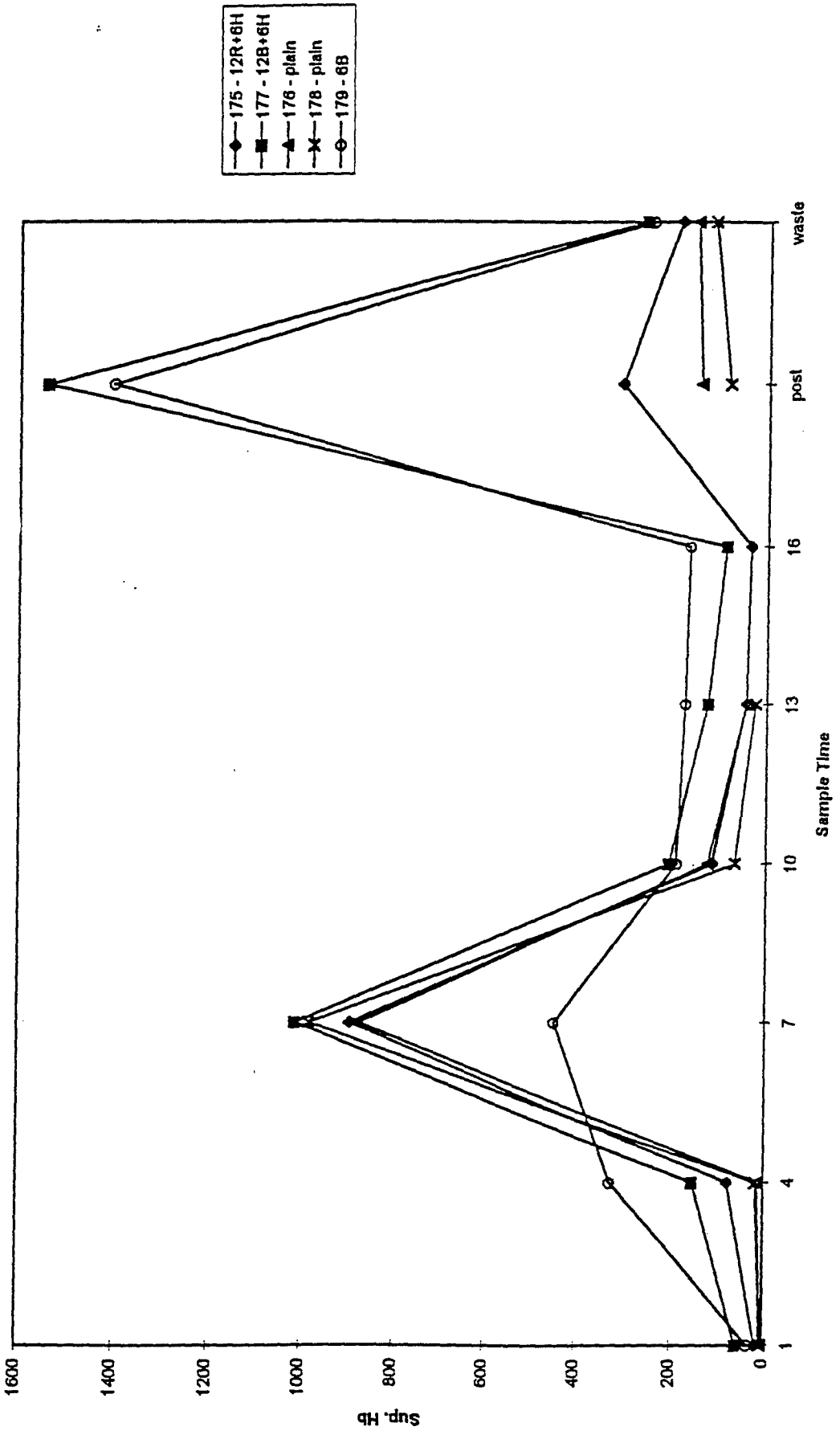
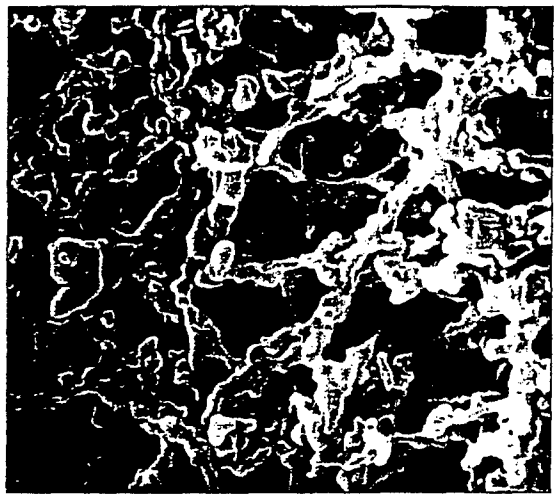
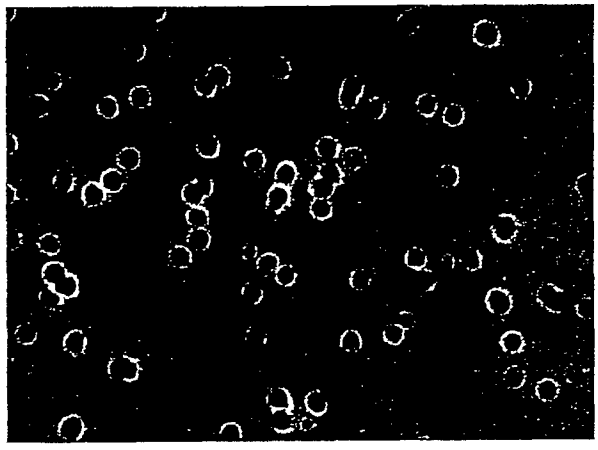


Figure 6: Supernatant Hb vs. Washing Time as Function of Rotor Type

Figure 7: Table of Filters



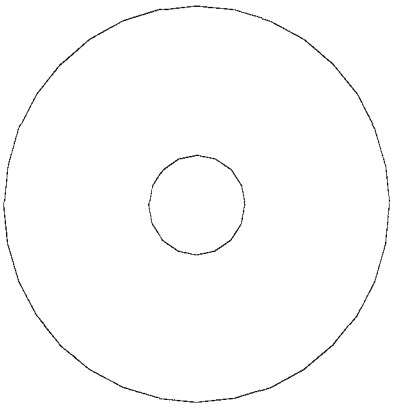
Depth Tortured Path Media



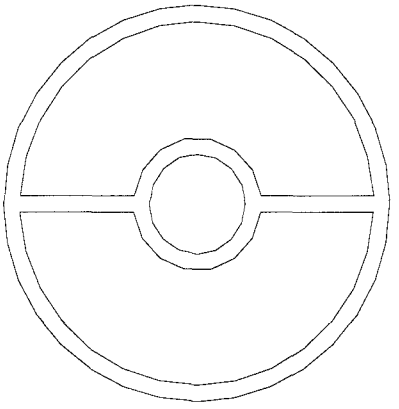
Microporous Media

Screen Media

Figure 8 - Full and Partial T-106



FULL T-106



PARTIAL T-106

Figure 9: Relationship Between Pressure and Recovery for Individual Units

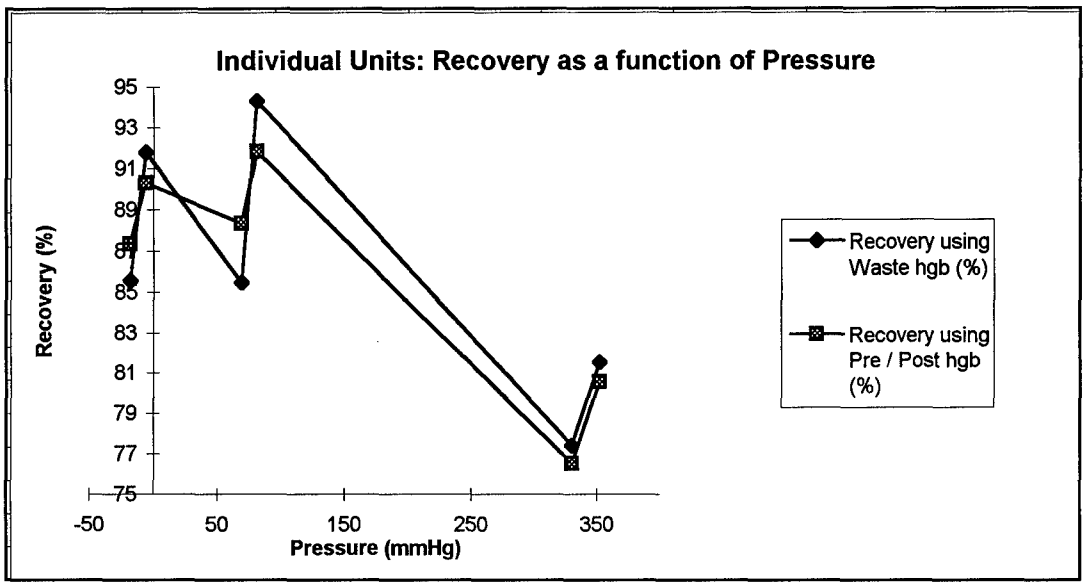


Figure 10: Filter 320 w/12R 6H Rotor - Rotor Speed vs Pressure and Plasma Hb at 100 and 160 mL/min Flowrates

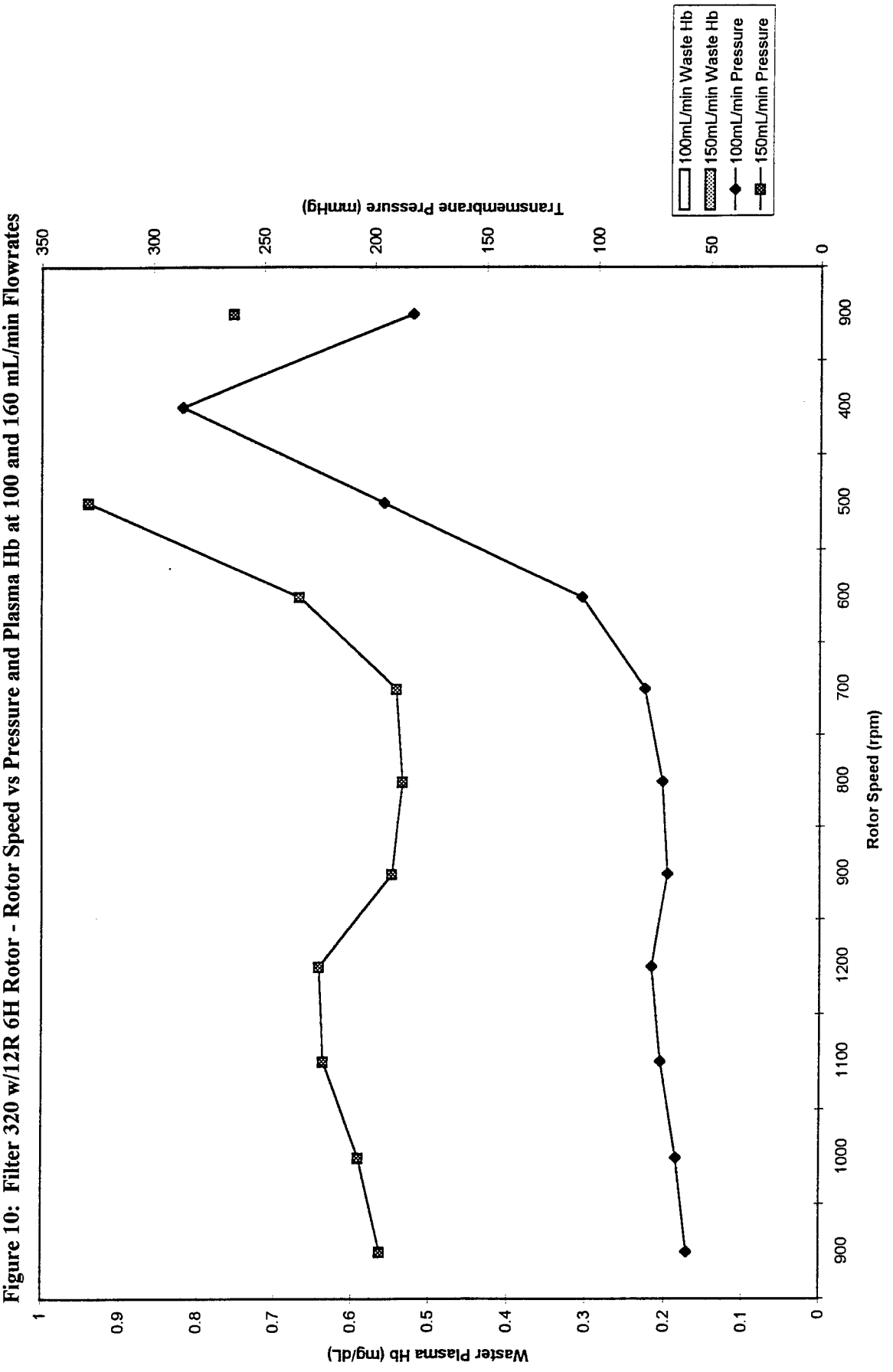


Figure 11 - Effects of Unit Size on Performance

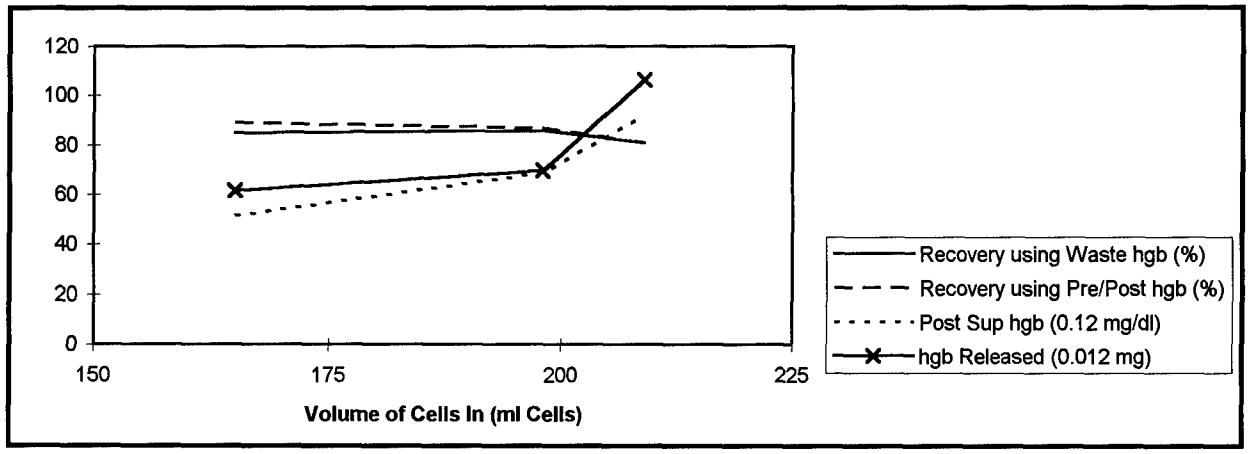


Figure 12 - Recirculation Configuration

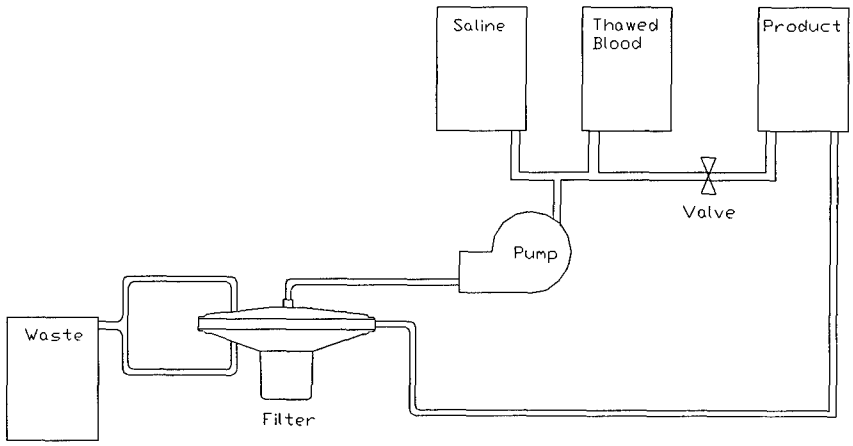


Figure 13 - Pre-Wash Degradation of Blood

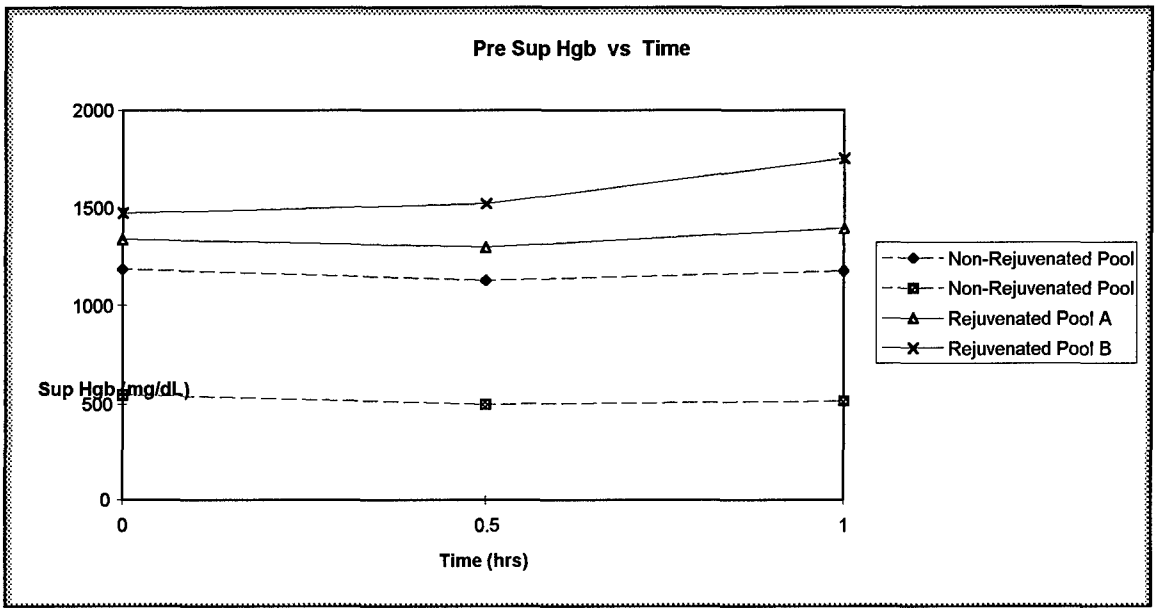


Figure 14 - Post-Wash Degradation of Blood

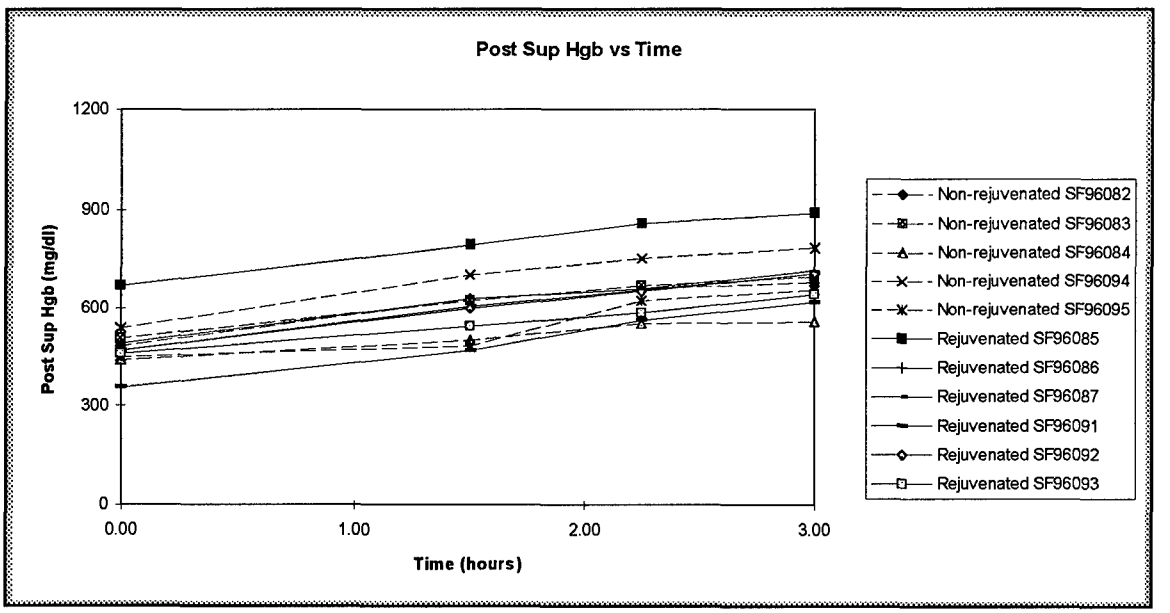


Figure 15 - Filter Volume at Variable Internal Filter Pressures

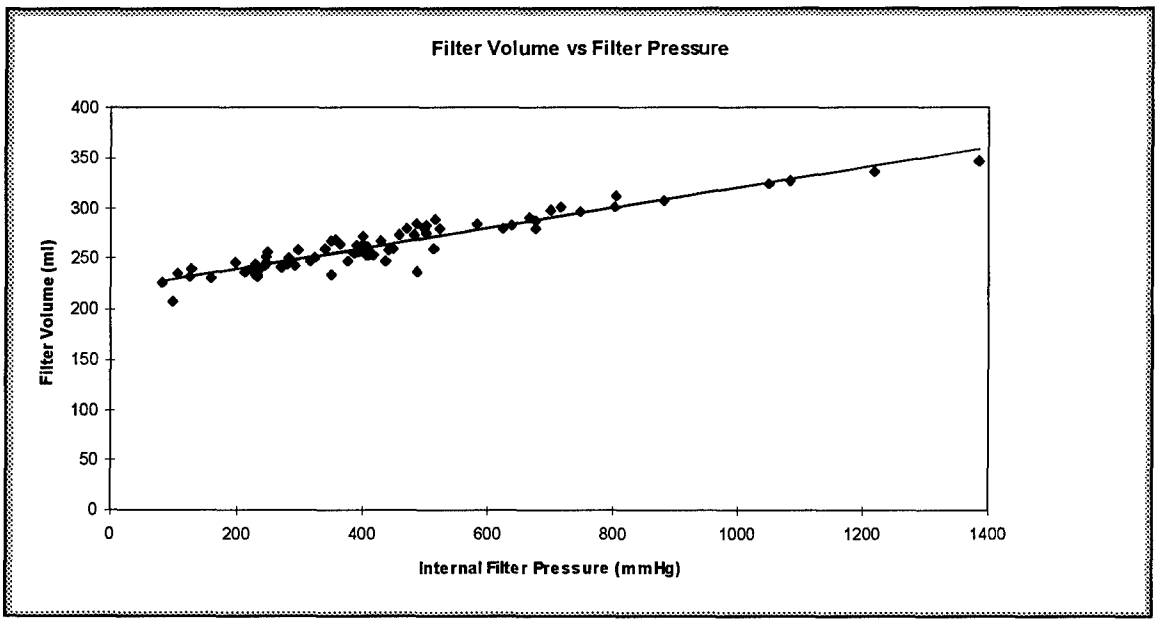
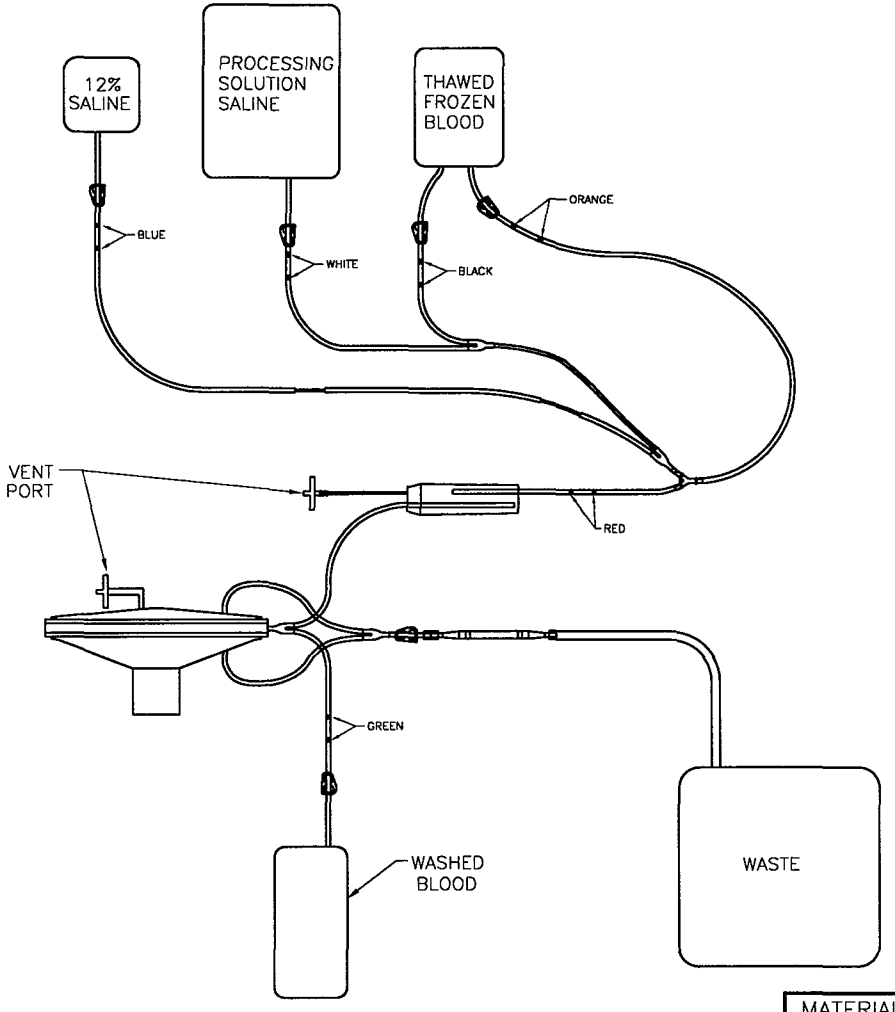


Figure 16: Fluid Circuit Attachments

R.C.N.	REV	CHANGE MADE	DATE:	APPD



F-14

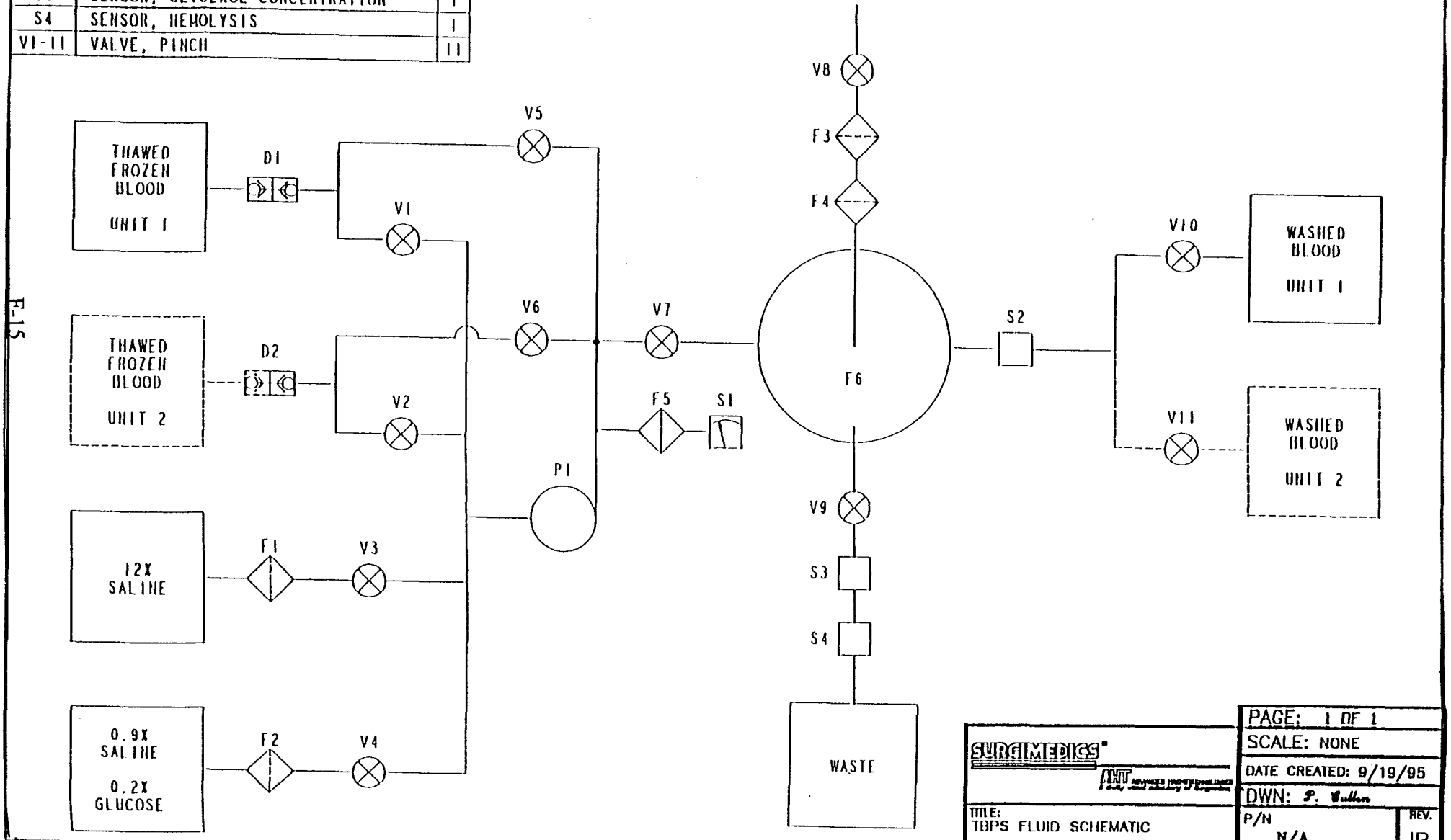
MATERIAL: N/A		SCALE: NONE
UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES DIMENSION LIMITS ONE PLACE OR FRACTION .X ±.030 TWO PLACES .XX ±.015 THREE PLACES .XXX ±.005 ANGULAR DIMENSIONS ±1/2° ENGINEERING DEPARTMENT APPROVAL REQUIRED ON SUBSTITUTIONS.	SURGIMEDICS® AHT ADVANCED HAEMOTECHNOLOGIES A wholly owned subsidiary of Surgimedics,	APPD: DATE CREATED: 4/7/97 DWN: A.SINGER
	TITLE: FILTER DISPOSABLES	P/N N/A — REV. IR

BACK-A.DWG

Figure 17: Fluid Schematic

SYMBOL	DESCRIPTION	QTY
D1-2	DOCKING DEVICE, STERILE	2
F1-2	FILTER, 0.22 MICRON HYDROPHILIC	2
F3-5	FILTER, 0.22 MICRON HYDROPHOBIC	3
F6	FILTER, TBPS	1
P1	PUMP, PERISTALTIC	1
S1	SENSOR, PRESSURE	1
S2	SENSOR, HEMATOCRIT	1
S3	SENSOR, GLYCEROL CONCENTRATION	1
S4	SENSOR, HEMOLYSIS	1
V1-11	VALVE, PINCH	11

BY	REV	DATE	APPR	ECO	DESCRIPTION
SMD	0.1	13 SEP 95		N/A	FIRST ISSUE
SMD	0.2	13 SEP 95		N/A	REVISED SYMBOLOGY
SMD	0.3	19 SEP 95		N/A	PER 14 SEP 95 DISCUSSION



F-15

SURGIMEDICS

TITLE: TBPS FLUID SCHEMATIC

P/N N/A

REV. IR

PAGE: 1 OF 1

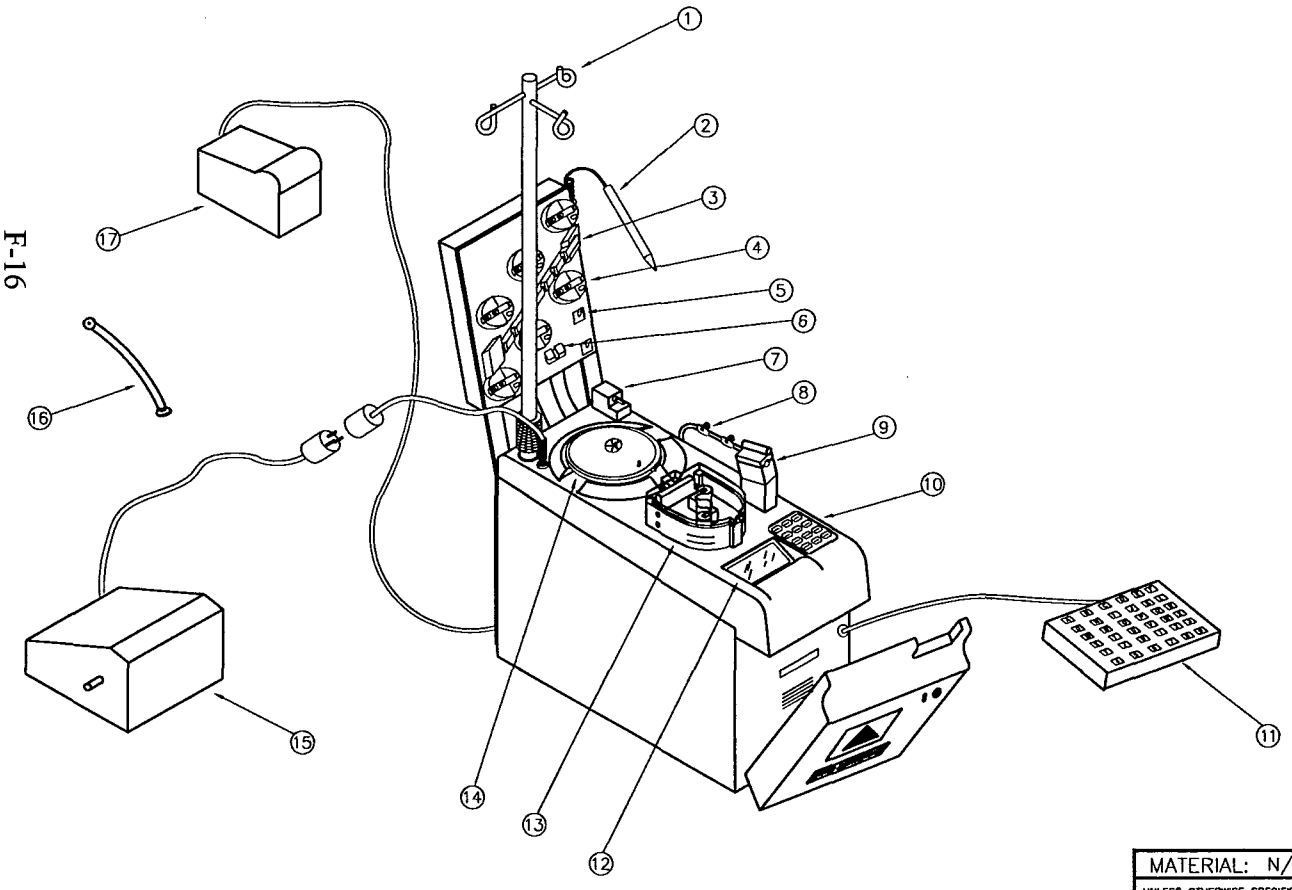
SCALE: NONE

DATE CREATED: 9/19/95

DWN: P. Wallen

Figure 18: Console Layout

R.C.N.	REV	CHANGE MADE	DATE:	APPD



ITEM	DESCRIPTION
1	SALINE AND PROCESS SOLUTION HANGER
2	BAR CODE SCANNER
3	TUBE HOLDER
4	PINCH VALVE
5	CHAMBER HOLDER
6	BUBBLE DETECTOR
7	PRESSURE SENSOR
8	WASTEBAG HOLDER
9	GLYCEROL & HEMOLYSIS DETECTOR
10	KEYPAD
11	KEYBOARD
12	DISPLAY
13	PERISTALTIC PUMP
14	FILTER
15	SHAKER
16	CALIBRATION TUBE
17	PRINTER

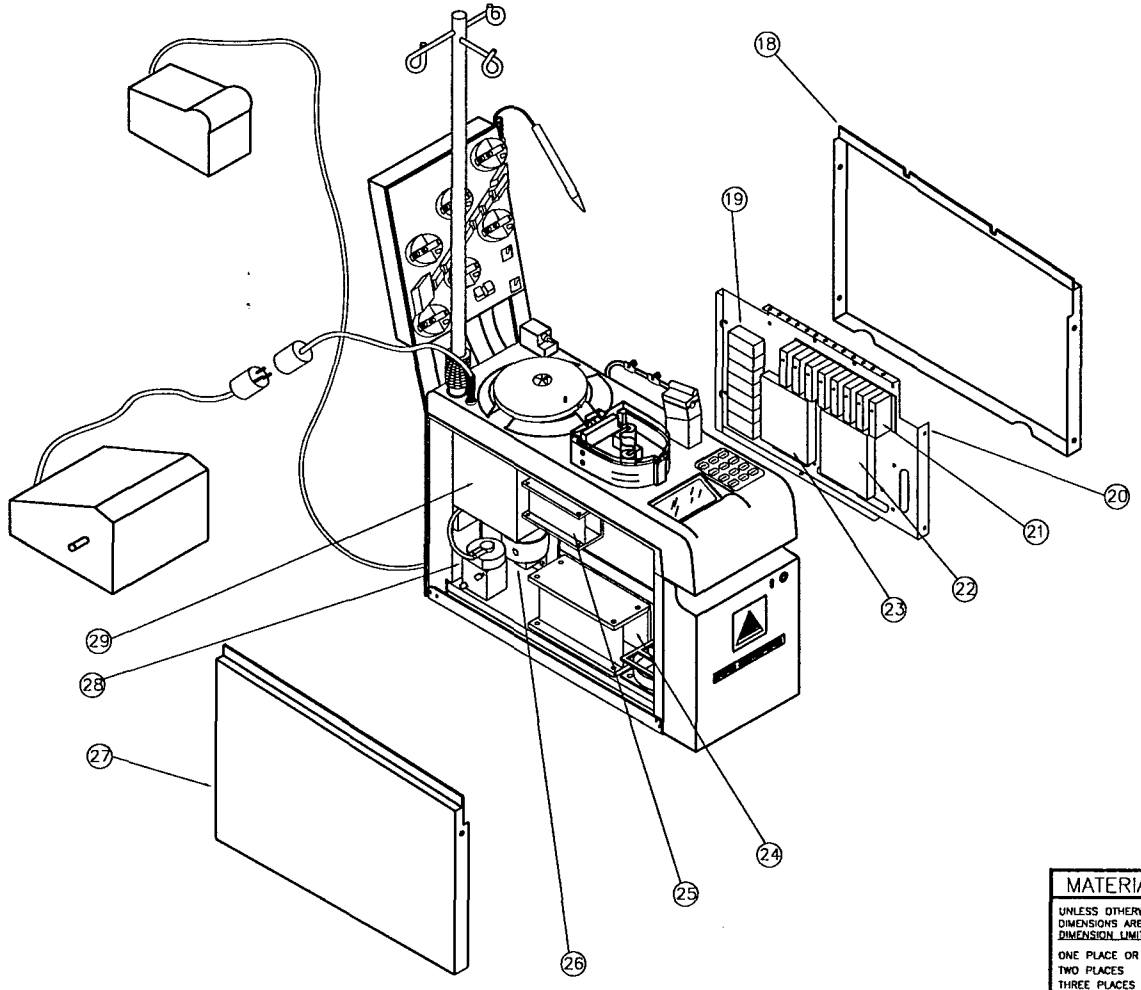
F-16

LAYOUT.DWG

MATERIAL: N/A		SCALE: N/A
UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES DIMENSION LIMITS ONE PLACE OR FRACTION .X ±.030 TWO PLACES .XX ±.015 THREE PLACES .XXX ±.005 ANGULAR DIMENSIONS ± 1/2° ENGINEERING DEPARTMENT APPROVAL REQUIRED ON SUBSTITUTIONS.	SURGIMEDICS® AHT ADVANCED HAEMOTECHNOLOGIES A wholly owned subsidiary of Surgimedics.	APPD: DATE CREATED: 4/14/97 DWN: A.SINGER
TITLE: TBPS LAYOUT PAGE 1 OF 2		P/N 490000-000 REV. 1R

Figure 19: Console Layout

R.C.N.	REV	CHANGE MADE	DATE:	APPD



ITEM	DESCRIPTION
18	SIDE PANEL, RIGHT
19	VALVES, PNEUMATIC
20	COMPONENT MOUNTING BOARD
21	I/O BOARD
22	OP AMP #1
23	OP AMP #2
24	10V POWER SUPPLY
25	5V POWER SUPPLY
26	MOTOR
27	SIDE PANEL, LEFT
28	PUMP, PNEUMATIC
29	RESERVIOR, PNEUMATIC

F-17

LAYOUT.DWG

MATERIAL: N/A		SCALE: N/A
UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES DIMENSION LIMITS ONE PLACE OR FRACTION .X ±.030 TWO PLACES .XX ±.015 THREE PLACES .XXX ±.005 ANGULAR DIMENSIONS ±1/2 ENGINEERING DEPARTMENT APPROVAL REQUIRED ON SUBSTITUTIONS.	SURGIMEDICS AHT ADVANCED HAEMOTECHNOLOGIES A wholly owned subsidiary of Surgimedics.	APPD: DATE CREATED: 4/14/97 DWN: A.SINGER
TITLE: TBPS LAYOUT PAGE 2 OF 2	P/N 490000-000	REV. IR

Figure 20: Tubing Loading Diagram I

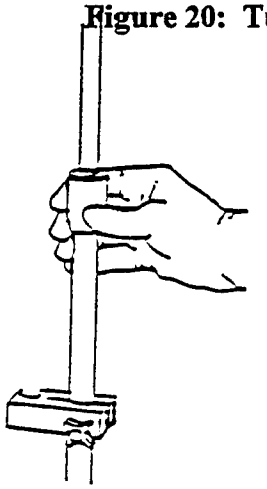


Figure 20A
Raise IV pole segments

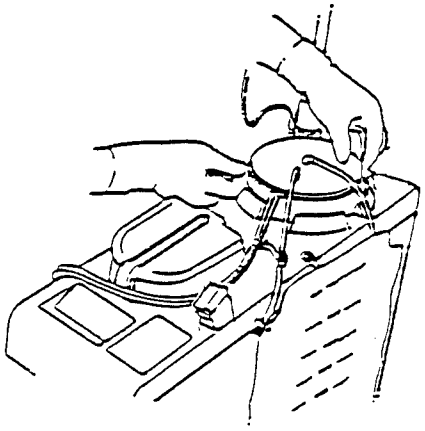


Figure 20B
Position filter

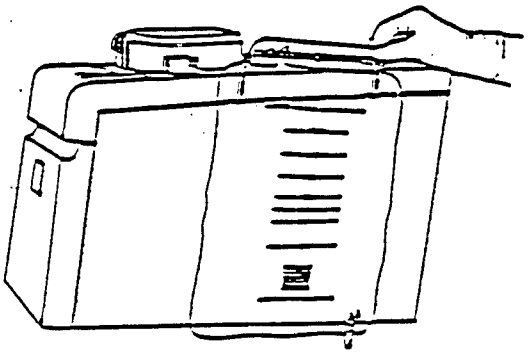


Figure 20C
Hang waste bag

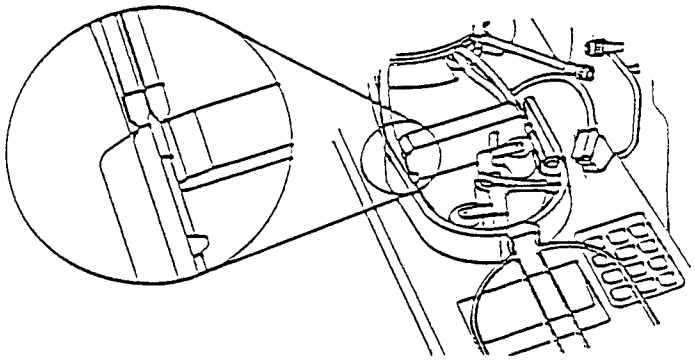


Figure 20D
Position pump segment

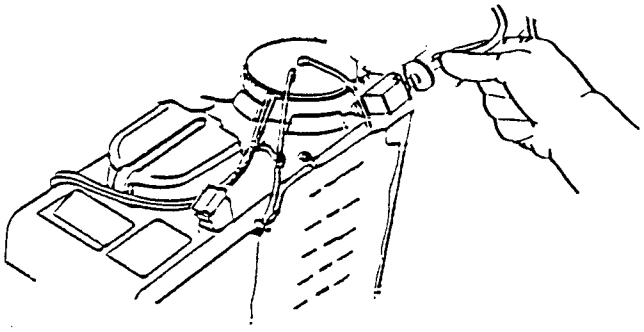


Figure 20E
Attach monitoring line

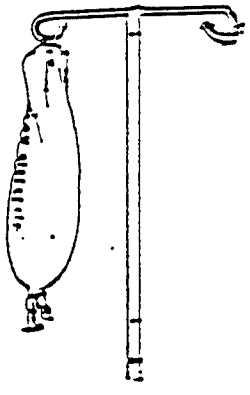


Figure 20F
Hang wash saline

Figure 21: Tubing Loading Diagram II

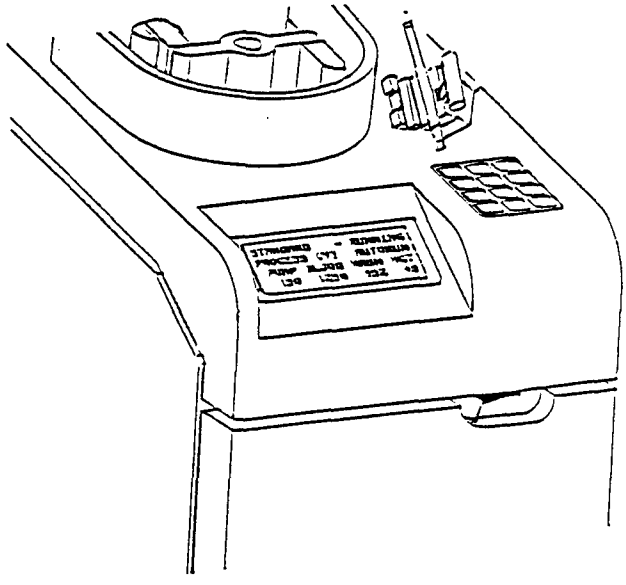


Figure 21A

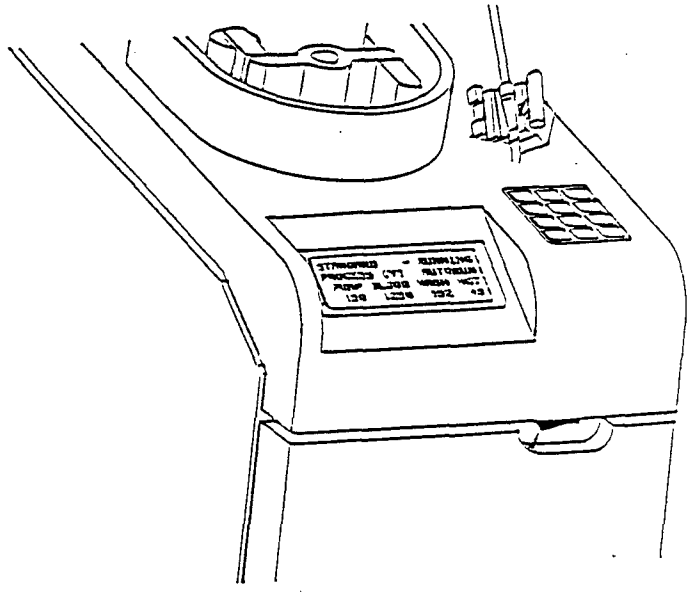


Figure 21C

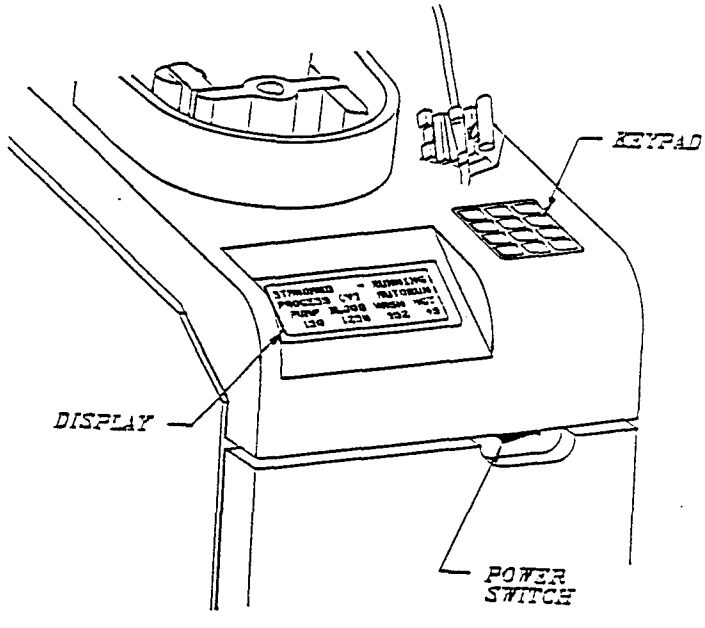


Figure 21B

Appendix A

Appendix A-1: Statistical Analysis of Process and Disposable Configuration Modification Results

Raw Data for Process and Disposable Configuration Modification Statistical Analysis

Modified	Unmodified	Unmodified w/3		Modified	Unmodified	Unmodified w/3		Modified	Unmodified	Unmodified w/3		Modified	Unmodified	Unmodified w/3
Recovery using Waste hgb				Recovery using Pre/Post hgb				Post Sup hgb				hgb Released		
61.9	65	72		44.8	73	64		220	345	155		8724	3399	3633
66.3	71	78		50.8	75	70		386	393	320		9829	5841	4375
68.2	71	82		63.3	78	75		422	403	348		10565	11962	4825
69.2	75	84		64.3	81	79		522	646	357		12121	12158	4854
71.8	75	86		65.8	83	79		556	747	385		12953	14018	5123
72.2	88	72		66.4	86	81		606	900	403		13405	16252	5304
72.8	90	83		68.7	88	81		642	943	411		14479	18035	5783
73.5		83		69		82		652		415		17258		5814
73.7		85		69.3		82		678		421				5914
73.9		81		70.1		82		723		431				5951
73.9		86		70.3		82		731		443				6081
74.3		79		70.5		82		882		455				6194
75.1		65		71.5		82		935		512				6435
75.5		76		71.9		82		1049		514				6877
76.9		84		73.9		83		1158		517				7053
		86				83				542				7369
		83				83				564				7590
		86				84				574				8225
		83				84				575				8234
		85				85				626				8352
		81				85				652				8855
		86				86				683				9952
		82				86				693				10255
		81				87				706				12156
		90				89				713				13860
		79				92				769				13914
		89				94				1063				20594

Anova: Single Factor for Recovery using Waste hgb

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	15	1079.2	71.94667	15.7841
Column 2	7	535	76.42857	85.28571
Column 3	27	2207	81.74074	30.12251

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	944.7301	2	472.3651	14.33414	1.45E-05	3.199588
Within Groups	1515.877	46	32.95384			
Total	2460.607	48				

Anova: Single Factor for Recovery using Pre/Post hgb

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	15	990.6	66.04	64.35971
Column 2	7	564	80.57143	30.95238
Column 3	27	2224	82.37037	35.08832

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2669.173	2	1334.587	30.71013	3.38E-09	3.199588
Within Groups	1999.047	46	43.45753			
Total	4668.22	48				

Anova: Single Factor for Post Sup hgb

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	15	10162	677.4667	63391.12
Column 2	7	4377	625.2857	62263.57
Column 3	23	10996	478.087	16604.45

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	388642.1	2	194321.1	5.018268	0.011111	3.219938
Within Groups	1626355	42	38722.74			
Total	2014997	44				

Anova: Single Factor for hgb Released

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	8	99334	12416.75	7543440
Column 2	7	81665	11666.43	28288509
Column 3	27	213572	7910.074	13574207

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.69E+08	2	84349313	5.716466	0.00665	3.2381
Within Groups	5.75E+08	39	14755500			
Total	7.44E+08	41				

Appendix A-2: Statistical Analysis of Rotor Design Results

Raw Data for Statistical Analysis of Rotor Design

Waste Recovery				Pre/Post Recovery				Post Sup hgb				hgb Released		
12	6	3		12	6	3		12	6	3		12	6	3
71	77	72		73	76	82		393	352	155		11962	7597	4854
71	78	76		78	82	82		646	415	357		12158	10602	7369
75	79	84		81	83	84		747	558	385		14018	11120	12156
75		85		83		92		900		683		16252		13914

t-Test: Paired Two Sample for Means - Waste Recovery		
	Variable 1	*Variable 2*
Mean	72.33333	78
Variance	5.333333	1
Observatio	3	3
Pearson C	0.866025	
Hypothesi	0	
df	2	
t Stat	-6.4254	
P(T<=t) on	0.011688	
t Critical o	2.919987	
P(T<=t) tw	0.023376	
t Critical t	4.302656	
t-Test: Paired Two Sample for Means - Pre/Post Recovery		
	Variable 1	*Variable 2*
Mean	78.75	85
Variance	18.91667	22.66667
Observatio	4	4
Pearson C	0.75659	
Hypothesi	0	
df	3	
t Stat	-3.90434	
P(T<=t) on	0.014917	
t Critical o	2.353363	
P(T<=t) tw	0.029834	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - Post sup hgb		
	Variable 1	*Variable 2*
Mean	671.5	395
Variance	45375	47362.67
Observatio	4	4
Pearson C	0.955329	
Hypothesi	0	
df	3	
t Stat	8.570811	
P(T<=t) on	0.001669	
t Critical o	2.353363	
P(T<=t) tw	0.003338	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - hgb released		
	Variable 1	*Variable 2*
Mean	13597.5	9573.25
Variance	3990070	17547582
Observatio	4	4
Pearson C	0.936152	
Hypothesi	0	
df	3	
t Stat	3.321677	
P(T<=t) on	0.022499	
t Critical o	2.353363	
P(T<=t) tw	0.044998	
t Critical t	3.182449	

Appendix A-3: Statistical Analysis of Filter Media Evaluation Results

Raw Data for Statistical Analysis of Media Evaluation Tests

Recovery using Waste hgb			Recovery using Pre/Post hgb			Post Sup hgb			hgb Released			Maximum Pressure	
1.2	3		1.2	3		1.2	3		1.2	3		1.2	3
86	86		85	87		564	493		5914	6267		157	208
82	87		84	89		403	235		8234	5677		170	272
81	81		83	85		652	421		7590	7471		212	289
90	87		86	83		517	463		4375	5693		232	353

t-Test: Paired Two Sample for Means - Waste Recovery		
	Variable 1	*Variable 2*
Mean	84.75	85.25
Variance	16.91667	8.25
Observati	4	4
Pearson C	0.599589	
Hypothesi	0	
df	3	
t Stat	-0.30151	
P(T<=t) on	0.391358	
t Critical o	2.353363	
P(T<=t) tw	0.782716	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - Pre/Post Recovery		
	Variable 1	*Variable 2*
Mean	84.5	86
Variance	1.666667	6.666667
Observati	4	4
Pearson C	-0.4	
Hypothesi	0	
df	3	
t Stat	-0.90453	
P(T<=t) on	0.216194	
t Critical o	2.353363	
P(T<=t) tw	0.432389	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - Post Sup hgb		
	Variable 1	*Variable 2*
Mean	534	403
Variance	10758	13416
Observati	4	4
Pearson C	0.716182	
Hypothesi	0	
df	3	
t Stat	3.139132	
P(T<=t) on	0.025849	
t Critical o	2.353363	
P(T<=t) tw	0.051698	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - hgb Released		
	Variable 1	*Variable 2*
Mean	6528.25	6277
Variance	3016895	708930.7
Observati	4	4
Pearson C	0.343696	
Hypothesi	0	
df	3	
t Stat	0.304655	
P(T<=t) on	0.39027	
t Critical o	2.353363	
P(T<=t) tw	0.780541	
t Critical t	3.182449	
t-Test: Paired Two Sample for Means - Maximum Pressure		
	Variable 1	*Variable 2*
Mean	192.75	280.5
Variance	1235.583	3552.333
Observati	4	4
Pearson C	0.921938	
Hypothesi	0	
df	3	
t Stat	-5.77066	
P(T<=t) on	0.005172	
t Critical o	2.353363	
P(T<=t) tw	0.010345	
t Critical t	3.182449	

Appendix A-4: Statistical Analysis of the Effects of Flow Rate on Hemolysis

Raw Data for Statistical Analysis of Effects of Flow Rate on Hemolysis

Description	Waste hgb Recovery	Pre/Post hgb Recovery	Post Sup. hgb (mg/dl)	Post Total Sup. hgb (mg)	Waste Total hgb (mg)	hgb Released (mg)	Processing Time (min)
FR=60	83	86	348	455	8280	5783	36
FR=60	79	83	626	626	8808	8352	37
FR=60	86	89	416	406	5633	5371	37
FR=60	87	92	287	249	5484	5101	36
FR=100	81	82	455	479	8516	6081	19
FR=100	89	94	320	284	4462	3633	23
FR=100	79	86	832	805	8925	9061	19
FR=100	87	89	818	581	5107	5056	22

Statistical Analysis of Effects of Flow Rate on Hemolysis

t-Test: Paired Two Sample for Means - Waste Recovery		
	Variable 1	*Variable 2*
Mean	83.75	84
Variance	12.91667	22.66667
Observatio	4	4
Pearson C	-0.44806	
Hypothesi	0	
df	3	
t Stat	-0.07007	
P(T<=t) on	0.474273	
t Critical o	2.353363	
P(T<=t) tw	0.948546	
t Critical t	3.182449	

t-Test: Paired Two Sample for Means - Pre/Post Recovery		
	Variable 1	*Variable 2*
Mean	87.5	87.75
Variance	15	25.58333
Observatio	4	4
Pearson C	-0.28076	
Hypothesi	0	
df	3	
t Stat	-0.06962	
P(T<=t) on	0.47444	
t Critical o	2.353363	
P(T<=t) tw	0.948879	
t Critical t	3.182449	

t-Test: Paired Two Sample for Means - Post Sup hgb		
	Variable 1	*Variable 2*
Mean	419.25	606.25
Variance	21774.25	66872.25
Observatio	4	4
Pearson C	-0.67388	
Hypothesi	0	
df	3	
t Stat	-0.99929	
P(T<=t) on	0.195648	
t Critical o	2.353363	
P(T<=t) tw	0.391297	
t Critical t	3.182449	

t-Test: Paired Two Sample for Means - hgb Released		
	Variable 1	*Variable 2*
Mean	6151.75	5957.75
Variance	2230241	5287656
Observatio	4	4
Pearson C	-0.64412	
Hypothesi	0	
df	3	
t Stat	0.112279	
P(T<=t) on	0.458847	
t Critical o	2.353363	
P(T<=t) tw	0.917694	
t Critical t	3.182449	

Appendix B: Media Investigation

COMPANY	MEDIA	NOTES
Depth		
Pall	1.2 μ Loprodyne Nylon 6,6	Tested extensively
	3.0 μ Loprodyne Nylon 6,6	Tested extensively
	1.2 μ Biodyne A Nylon 6,6	Tested extensively - similar to Loprodyne
	3.0 μ Biodyne A Nylon 6,6	Tested extensively - similar to Loprodyne
Gore	1.0 μ PolyTetraFlouroEthylene	Can't make hydrophilic
	3.0 μ PolyTetraFlouroEthylene	Can't make hydrophilic
MSI	1.2 μ Acetate Plus	Similar to Loprodyne
	1.2 μ MAGNA Nylon	Similar to Loprodyne
Texel	3.0 μ Felt with Polypropylene backing	Media has too much depth (1/4 in.)
Gelman Sciences	1.2 μ Supor Polyethersulfone	Similar to Loprodyne
3M media	1.0 μ High Density Polypropylene	Demand too high to get
Hollingsworth and Vose	Glass fiber in Acrylic resin media	Not USP or FDA approved
Microporous		
Millipore	1.2 μ Isopore Polycarbonate	Tested extensively - Static makes difficult to manufacture
	2.0 μ Isopore Polycarbonate	Tested - pore size too large
	3.0 μ Isopore Polycarbonate	Tested - pore size too large
Whatman	1.0 μ Cyclopore Polycarbonate	Tested extensively - Static makes difficult to manufacture
	2.0 μ Cyclopore Polycarbonate	Tested - pore size too large
	3.0 μ Cyclopore Polycarbonate	Tested - pore size too large
	1.0 μ Cyclopore Polyester	Tested extensively - easy to manufacture and gives good results
	2.0 μ Cyclopore Polyester	Tested - pore size too large
	3.0 μ Cyclopore Polyester	Tested - pore size too large
	0.2 μ Anopore Aluminum Oxide	Very brittle - too small pore size
Screen		
Tetko	5 μ Pecap Polyester	Tested - pore size too large
	5 μ Nitex Nylon	Tested - pore size too large
Porex	7.0 μ minimum Polyester	Media not approved for patient infusion
Synthetic Technologies	10.0 μ minimum media	Pore size too large
Interflo	10.0 μ minimum media	Pore size too large
ALSOP	Large pore media for air filtration	Pore size too large
Columbia Filter Co.	Large pore media for air filtration	Pore size too large
Ertel	Large pore media for air filtration	Pore size too large
Saati	7.0 μ minimum media	Pore size too large
PTI	0.5 to 5.0 μ Stainless Steel	minimum cost is $75 per sq. ft.

Appendix C

Appendix C, Table 1: Pall 3.0um LP Bonded with Partial and Full T-106 Support

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96009	7/18/96	3R 6H	3 LP partial	27	550	211	347	983	558	2093	12177	75.5	71.4	1092	48
SF96013	7/19/96	3R 6H	3 LP partial	23	306	157	293	543	217	2136	4308	88.9	87.5	181	51
SF96015	7/22/96	3R 6H	3 LP partial	24	214	158	310	967	199	2088	3692	89.1	81.3	436	53
SF96023	7/25/96	3R 6H	3 LP partial	24	249	163	331	617	187	2154	3886	89.3	77.5	456	55
SF96010	7/18/96	3R 6H	3 LP full	27	609	211	347	983	544	2095	11777	77.3	76.7	1000	49
SF96012	7/19/96	3R 6H	3 LP full	23	269	156	291	539	211	2111	4603	87.8	83.9	561	49
SF96016	7/22/96	3R 6H	3 LP full	24	270	158	310	967	160	2106	2855	91.4	86.4	389	54
SF96022	7/25/96	3R 6H	3 LP full	24	319	163	331	617	289	2126	6461	83.5	74.2	771	50

All Operating Parameters are 800 rpm, 100mL/min, with 3R 6H rotors

Appendix C, Table 2: Pall 3.0 um LP and 3.0 um BA with Full T-106 Support

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96034	7/31/96	3R 6H	3 LP	23	402	167	338	588	200	2124	4316	89.3	85.1	518	52
SF96039	8/1/96	3R 6H	3 LP	24	231	154	330	2912	333	2125	4744	83	80.9	454	48
SF96046	8/5/96	3R 6H	3 LP	24	398	172	316	510	182	2108	4202	90	85	772	56
SF96055	8/8/96	old 3R6H	3 LP	23	519	180	285	611	181	2077	3758	91	83	560	58
SF96035	7/31/96	3R 6H	3 BA	23	228	167	338	588	200	2135	4280	89.3	84.1	602	58
SF96041	8/1/96	3R 6H	3 BA	24	114	156	334	2947	411	2153	6355	79	74.4	377	49
SF96048	8/5/96	3R 6H	3 BA	24	240	164	302	487.38	100	2116	1995	95	92	395	61
SF96057	8/8/96	old 3R6H	3 BA	23	420	180	285	611	182	2073	3624	92	87	457	60

All Operating Parameters are 800 rpm, 100 mL/min, with 3R 6H rotors

Appendix C, Table 3: Pall 1.2 um LP and 1.2 um BA with Full T-106 Support

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96036	7/31/96	3R 6H	1.2 BA	23	234	167	338	588	242	2124	5276	87.1	81.9	642	54
SF96052	8/7/96	old 3R6H	1.2 BA	23	460	180	285	2760	306	2048	4954	86	82	1191	58
SF96056	8/8/96	old 3R6H	1.2 BA	23	426	180	285	611	170	2064	3416	92	86	510	60
SF96037	7/31/96	3R 6H	1.2 LP	23	251	167	338	588	253	2127	5232	87.6	85.4	419	57
SF96050	8/7/96	old 3R6H	1.2 LP	24	447	180	285	2760	331	2075	5319	85	79	1197	59
SF96054	8/8/96	old 3R6H	1.2 LP	23	412	180	285	611	186	2015	3865	91	86	717	60

All Operating Parameters are 800 rpm, 100 mL/min, with 3R 6H rotors

Appendix C, Table 4: Pall 3.0 um LP and Whatman 1.0 um Cyclopore PC with full T-106 Support

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96064	8/15/96	old 3R6H	3 LP	23	391	148	265	1840	405	2053	7384	79	78	616	45
SF96079	8/21/96	3R6H	3 LP	23	503	173	280	1291	218	2061	3816	90	86	535	57
SF96099	9/10/96	3R6H	3 LP	23	677	173	270	2430	433	2032	7080	81	80	509	50
SF96102	9/11/96	3R6H	3 LP	23	1050	200	286	1543	413	2000	7600	84	81	524	48
SF96108	9/12/96	3R6H	3 LP	23	675	178	314	1384	298	2058	5444	87	81	527	54
SF96065	8/15/96	old 3R6H	1 Cycl. PC	23	157	148	265	1840	335	2050	5615	83	85	551	54
SF96077	8/21/96	3R6H	1 Cycl. PC	23	299	173	280	1291	227	2070	3946	90	83	583	61
SF96100	9/10/96	3R6H	1 Cycl. PC	23	660	173	270	2430	467	2028	7640	80	80	398	48
SF96103	9/11/96	3R6H	1 Cycl. PC	23	1360	200	286	1543	459	1947	8400	83	80	498	43
SF96109	9/12/96	3R6H	1 Cycl. PC	23	684	178	314	1384	292	2057	5224	88	85	408	51

All Operating Parameters are 800 rpm, 100 mL/min, with 3R 6H rotors

Appendix C, Table 5: Pall 3.0 um LP and Whatman 1.0 um Cyclopore PET with Full T-106 Support

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96064	8/15/96	old 3R6H	3 LP	23	391	148	265	1840	405	2053	7384	79	78	616	45
SF96071	8/16/96	old 3R6H	3 LP	23	1220	185	313	1878	391	2013	7363	83	79	740	45
SF96075	8/19/96	old 3R6H	3 LP	24	805	190	330	754	238	2014	4659	90	89	424	53
SF96079	8/21/96	3R6H	3 LP	23	503	173	280	1291	218	2061	3816	90	86	535	57
SF96099	9/10/96	3R6H	3 LP	23	677	173	270	2430	433	2032	7080	81	80	509	50
SF96102	9/11/96	3R6H	3 LP	23	1050	200	286	1543	413	2000	7600	84	81	524	48
SF96105	9/11/96	3R6H	3 LP	23	884	178	300	2893	517	2042	8427	79	74	459	46
SF96108	9/12/96	3R6H	3 LP	23	675	178	314	1384	298	2058	5444	87	81	527	54
SF96066	8/15/96	old 3R6H	1 PET Support	23	90	148	265	1840	317	2082	5203	83	82	434	55
SF96070	8/16/96	old 3R6H	1 PET Support	23	322	185	313	1878	289	2115	4964	87	83	879	66
SF96073	8/19/96	old 3R6H	1 PET Support	24	345	190	330	754	208	2126	4230	91	87	659	66
SF96076	8/21/96	3R6H	1 PET Support	23	273	173	280	1291	194	2083	3287	91	84	687	66
SF96101	9/10/96	3R6H	1 PET Support	23	760	173	270	2430	357	2008	5396	84	82	396	47
SF96104	9/11/96	3R6H	1 PET Support	23	1014	200	286	1543	401	2009	7851	83	79	763	46
SF96107	9/11/96	3R6H	1 PET Support	23	895	178	300	2893	524	2022	8345	79	74	360	44
SF96110	9/12/96	3R6H	1 PET Support	23	426	178	314	1384	197	2073	3175	91	81	378	54

All Operating Parameters are 800 rpm, 100 mL/min, with 3R 6H rotors

Appendix C, Table 6: Pall 3.0 um LP and Unsupported Whatman 1.0 um Cyclopore PET

Test #	Date of Test	Rotor Type	Filter Media	Washing Time (min)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Weight of Blood In (g)	Pre Total Sup. hgb / unit (mg)	Waste Sup. hgb (mg/dl)	Waste Volume (ml)	hgb Released (mg)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Sup. hgb (mg/dl)	Post Hematocrit
SF96112	9/13/96	3R6H	3 LP	24	1386	191	298	2165	471	1987	8706	81	79	753	42
SF96120	9/18/96	3R6H	3 LP	23	667	180	288	608	261	2014	5228	88	86	408	51
SF96122	9/19/96	3R6H	3 LP	23	748	180	270	573	220	2020	4660	90	87	532	50
SF96124	9/19/96	3R6H	3 LP	23	803	180	277	879	316	2028	6448	86	84	587	48
sf6113-5	9/14/96	3R6H	1 Cycl. PET us	23.0	329.3	191.1	298.0	2165.0	339.0	2088.7	5335.8	86.8	83.9	567.0	67.7
SF96121	9/18/96	3R6H	1 Cycl. PET us	23	276	180	288	608	153	2079	2850	93	88	339	66
SF96123	9/19/96	3R6H	1 Cycl. PET us	23	254	180	270	573	167	2067	3186	93	89	375	65
SF96125	9/19/96	3R6H	1 Cycl. PET us	23	295	180	277	879	191	2057	3453	92	88	475	65

All Operating Parameters are 800 rpm, 100 mL/min, with 3R 6H rotors

Appendix C, Table 7: Effect of Rotor Speed on Performance of TBPS Configured with Plain Rotor

									Post Values (Taken after Processing)			
									Goal >=70	Goal <150	Goal >80%	Goal >80%
Test #	Date of Test	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Pre Sup. hgb (mg/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
SF96141	10/10/96	900	500	180	22.2	3751	401	287	53	382	83	83
SF96155	10/16/96	1000	418	194	22.10	3089	348	278	60	248	87	84
			459.0	**186.9**	**22.2**	**3420.0**	**374.5**	**282.5**	**56.5**	**315.0**	**85.0**	**83.6**
SF96140	10/10/96	1200	183	180	22.2	3751	419	248	62	274	83	83
SF96142	10/10/96	1200	164	180	22.2	3751	340	246	63	214	86	84
SF96154	10/16/96	1200	289	194	22.10	3089	314	256	66	401	88	86
SF96158	10/16/96	1200	326	195	22.30	4069	433	270	66	531	84	82
SF96167	10/25/96	1200	315	195	21.50	2149	307	259	67	525	88	83
SF96169	10/29/96	1200	252	195	23.10	3060	338	249	67	554	87	85
			254.8	**189.8**	**22.2**	**3311.5**	**358.5**	**254.7**	**65.2**	**416.5**	**85.9**	**83.8**
SF96153	10/16/96	1400	196	194	22.10	3089	317	249	67	286	88	85
SF96160	10/24/96	1400	274	195	22.30	4069	329	261	68	391	87	79
SF96165	10/25/96	1400	187	195	21.50	2149	378	254	67		84	78
SF96171	10/29/96	1400	177	195	23.10	3060	369	240	69	350	86	84
			208.5	**194.7**	**22.3**	**3091.8**	**348.3**	**251.0**	**67.8**	**342.3**	**86.3**	**81.7**
SF96139	10/10/96	1500	140	180	22.2	3751	424	214	70	323	82	80
SF96144	10/10/96	1500	118	180	22.2	3751	503	231	64	289	80	79
			129.0	**180.1**	**22.2**	**3751.0**	**463.5**	**222.5**	**67.0**	**306.0**	**81.0**	**79.5**
SF96152	10/16/96	1600	156	194	22.10	3089	409	240	68	321	84	82
SF96162	10/24/96	1600	244	195	22.30	4069	472	254	67	240	83	77
SF96163	10/25/96	1600	215	195	21.50	2149	359	250	70	490	86	82
SF96173	10/29/96	1600	157	195	23.10	3060	406	238	68	313	85	82
			193.0	**194.7**	**22.3**	**3091.8**	**411.5**	**245.5**	**68.3**	**341.0**	**84.4**	**80.9**
SF96143	10/10/96	1800	126	180	22.2	3751	640	218	62	164	73	71
SF96145	10/10/96	1800	129	180	22.2	3751	797	220	57	255	67	66
SF96151	10/16/96	1800	137	194	22.10	3089	480	230	69	325	81	79
			130.7	**184.6**	**22.2**	**3530.3**	**639.0**	**222.7**	**62.7**	**248.0**	**74.0**	**72.1**

Appendix C, Table 8: Effect of Rotor Speed on Performance of TBPS Configure with Three Ridge Rotor

									Post Values (Taken after Processing)			
									Goal >=70	Goal <150	Goal >80%	Goal >80%
Test #	Date of Test	Rotor Speed (RPM)	Maximum Pressure (mmHg)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Pre Sup. hgb (mg/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
SF96157	10/24/96	800	386	195	22.30	4069	423	270	65	488	85	81
SF96168	10/25/96	800	350	195	21.50	2149	329	264	68	795	87	84
SF96170	10/29/96	800	285	195	23.10	3060	334	258	63	752	87	87
			340.3	**195.0**	**22.3**	**3092.7**	**362.0**	**264.0**	**65.3**	**678.3**	**86.2**	**84.1**
SF96159	10/24/96	1000	268	195	22.30	4069	426	256	69	517	84	81
SF96166	10/25/96	1000	245	195	21.50	2149	308	244	72	929	87	81
SF96172	10/29/96	1000	184	195	23.10	3060	334	237	71	402	87	85
			232.3	**195.0**	**22.3**	**3092.7**	**356.0**	**245.7**	**70.7**	**616.0**	**86.2**	**82.2**

Appendix C, Table 9: Evaluation of Use of Magnet Seal on Performance of TBPS

			Pre Values (Taken after Thaw and Prior to any Dilution)							Goal >=70		Post Values (Taken after Processing) Goal <150	Goal >80%	Goal >80%	Goal<400
Seal diameter	Test #	Date of Test	Maximum Pressure (mmHg)	Weight of Blood In (g)	Hematocrit of Blood In (%)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Total Hgb (g/dl)	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Osmo (mOs/kg H2O)
none	SF96176	11/18/96	208	304	74	195	22.20	320	250	68	20.9	391	87	84	242
none	SF96181	11/18/96	217	290	78	195	24.10	440	252	67	20.4	464	84	80	230
none	SF96182	11/19/96	135	274	76	180	22.70	314	237	66	20.1	268	87	83	279
none	SF96187	11/19/96	132	277	75	180	22.40	275	234	67	20.7	231	88	85	168
0.005	SF96177	11/18/96	210	304	74	195	22.20	504	237	63	19.3	480	80	73	267
0.005	SF96180	11/18/96	238	290	78	195	24.10	503	246	65	19.1	575	80	73	267
0.005	SF96183	11/19/96	150	274	76	180	22.70	410	229	64	19.6	405	83	78	291
0.005	SF96186	11/19/96	148	277	75	180	22.40	342	231	65	20.5	317	86	83	193
0.010	SF96178	11/18/96	229	304	74	195	22.20	397	243	65	19.7	517	84	77	242
0.010	SF96179	11/18/96	260	290	78	195	24.10	453	248	65	20.2	689	82	77	304
0.010	SF96184	11/19/96	158	274	76	180	22.70	517	227	62	18.6	430	78	74	254
0.010	SF96185	11/19/96	145	277	75	180	22.40	375	232	64	20.0	329	84	81	230
control	**average**		**173.0**	**286.3**	**75.8**	**187.6**	**22.9**	**337.3**	**243.3**	**67.0**	**20.5**	**338.5**	**86.6**	**82.7**	**229.8**
	stdev		**45.8**	**13.7**	**1.7**	**8.7**	**0.9**	**71.3**	**9.1**	**0.8**	**0.4**	**108.1**	**2.1**	**2.2**	**46.1**
0.005	**average**		**186.5**	**286.3**	**75.8**	**187.6**	**22.9**	**439.8**	**235.8**	**64.3**	**19.6**	**444.3**	**82.0**	**76.7**	**254.5**
	stdev		**44.8**	**13.7**	**1.7**	**8.7**	**0.9**	**78.7**	**7.6**	**1.0**	**0.6**	**109.7**	**2.8**	**4.7**	**42.5**
	p-value		**0.688**	**1.000**	**1.000**	**1.000**	**1.000**	**0.102**	**0.253**	**0.005**	**0.045**	**0.219**	**0.042**	**0.060**	**0.460**
0.01	**average**		**198.0**	**286.3**	**75.8**	**187.6**	**22.9**	**435.5**	**237.5**	**64.0**	**19.6**	**491.3**	**82.1**	**77.1**	**257.5**
	stdev		**55.4**	**13.7**	**1.7**	**8.7**	**0.9**	**63.5**	**9.7**	**1.4**	**0.7**	**152.6**	**2.8**	**3.1**	**32.5**
	p-value		**0.513**	**1.000**	**1.000**	**1.000**	**1.000**	**0.085**	**0.419**	**0.010**	**0.064**	**0.153**	**0.045**	**0.025**	**0.363**

Appendix C, Table 10: Evaluation of Thawed Blood Diluents with TBPS Washing

			Pre Values (Taken after Thaw and Prior to any Dilution)							Goal >=70		Post Values (Taken after Processing) Goal <150	Goal >80%	Goal >80%	Goal<400
Diluents Used	Test #	Date of Test	Maximum Pressure (mmHg)	Weight of Blood In (g)	Hematocrit of Blood In (%)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Total Hgb (g/dl)	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Osmo (mOs/kg H2O)
12/0.9	SF96188	11/21/96	143	293	70	178	22.00	386	239	66	20.6	238	85	82	230
12/0.9	SF96191	11/22/96	163	297	68	176	22.10	490	248	61	19.1	253	80	78	193
12/0.9	SF96192	11/22/96	258	293	77	195	23.60	410	261	67	20.5	495	85	84	279
12/0.9	SF96194	11/25/96	150	265	81	185	23.30	387	242	67	20.0	367	85	85	242
12/0.9	SF96196	11/25/96	166	265	81	185	23.30	283	243	68	21.0	351	89	90	230
12/0.9	SF96198	12/2/96	190	304	70	185	21.20	238	250	68	21.5	382	91	90	230
12/0.9	SF96202	12/3/96	196	304	70	185	21.20	457	241	67	20.2	319	82	81	193
12/0.9	SF96206	12/4/96	146	296	72	185	21.10	372	239	68	20.7	238	85	86	180
8.5/1.6	SF96189	11/21/96	160	293	70	178	22.00	595	235	63	19.0	374	76	75	230
8.5/1.6	SF96190	11/22/96	137	296	68	175	22.10	641	251	59	17.8	364	75	74	217
8.5/1.6	SF96193	11/22/96	218	293	77	195	23.60	735	254	63	18.0	311	73	72	193
8.5/1.6	SF96195	11/25/96	136	265	81	185	23.30	591	230	64	18.7	396	76	76	242
8.5/1.6	SF96197	11/25/96	141	265	81	185	23.30	461	241	64	19.1	248	81	81	193
8.5/1.6	SF96201	12/3/96	177	304	70	185	21.20	498	242	66	20.1	290	81	81	205
8.5/1.6	SF96203	12/3/96	150	304	70	185	21.20	485	238	67	20.0	238	81	80	168
8.5/1.6	SF96204	12/4/96	170	296	72	185	21.10	381	246	67	20.6	242	85	88	217
12/0.9	**Average**		**176.50**	**289.63**	**73.63**	**184.27**	**22.23**	**377.88**	**245.38**	**66.50**	**20.45**	**330.38**	**85.17**	**84.45**	**222.13**
	StDev		**38.27**	**15.78**	**5.26**	**5.63**	**1.04**	**83.34**	**7.46**	**2.33**	**0.72**	**88.41**	**3.27**	**4.03**	**32.26**
8.5/1.6	**Average**		**161.13**	**289.50**	**73.63**	**184.19**	**22.23**	**548.38**	**242.13**	**64.13**	**19.16**	**307.88**	**78.59**	**78.28**	**208.13**
	StDev		**27.54**	**15.72**	**5.26**	**5.76**	**1.04**	**113.14**	**8.03**	**2.64**	**1.01**	**63.72**	**4.25**	**5.21**	**23.49**
	P value		**0.374**	**0.988**	**1.000**	**0.980**	**1.000**	**0.005**	**0.416**	**0.078**	**0.012**	**0.569**	**0.004**	**0.020**	**0.339**

Appendix C, Table 11: Effects of Warmed vs Room Temperature Saline on TBPS Performance

			Pre Values (Taken after Thaw and Prior to any Dilution)							Goal >=70	Post Values (Taken after Processing) Goal <150	Goal >80%	Goal >80%
Goal of Test	Test #	Date of Test	Maximum Pressure (mmHg)	Weight of Blood In (g)	Hematocrit of Blood In (%)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Total Hgb (g/dl)	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
unwarm	SF96209	12/9/96	155	289	74	185	20.80	301	232	66	19.6	375	87	82
unwarm	SF96211	12/9/96	119	278	77	185	22.30	403	238	65	19.4	498	83	81
unwarm	SF96213	12/10/96	143	292	73	185	21.10	430	237	66	20.6	229	83	86
unwarm	SF96215	12/11/96	120	268	80	185	23.50	599	236	63	18.7	396	77	76
			134.3	281.8	76.0	185.0	21.9	433.3	235.8	65.0	19.6	374.5	82.5	81.1
			17.7	11.0	3.2	0.2	1.2	123.7	2.6	1.4	0.8	110.9	4.2	3.9
warm	SF96210	12/9/96	116	289	74	185	20.80	278	232	70	19.9	373	88	83
warm	SF96212	12/9/96	121	278	77	185	22.30	260	240	69	21.0	353	89	88
warm	SF96214	12/10/96	124	292	73	185	21.10	320	239	68	21.2	165	88	89
warm	SF96216	12/11/96	150	268	80	185	23.50	596	240	65	18.4	322	77	76
	Average		**127.8**	**281.8**	**76.0**	**185.0**	**21.9**	**363.5**	**237.8**	**68.0**	**20.1**	**303.3**	**85.4**	**84.0**
	St. deviation		**15.2**	**11.0**	**3.2**	**0.2**	**1.2**	**157.0**	**3.9**	**2.2**	**1.3**	**94.5**	**5.7**	**5.8**
	P value		**0.598**	**1.000**	**1.000**	**1.000**	**1.000**	**0.513**	**0.429**	**0.066**	**0.498**	**0.367**	**0.449**	**0.444**

Appendix C, Table 12: Comparison of Processing Modifications to Evaluate Improve Mixing and Blood Circulation

			Pre Values (Taken after Thaw and Prior to any Dilution)							Goal >=70	Post Values (Taken after Processing)			
											Goal <150	Goal >80%	Goal >80%	
Goal of Test	Test #	Date of Test	Maximum Pressure (mmHg)	Weight of Blood In (g)	Hematocrit of Blood In (%)	Volume of RBCs In (ml)	Pre Total hgb (g/dl)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Total Hgb (g/dl)	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)
control	SF96217	12/11/96	95	292	73	185	21.30	217	236	70	22.0	318	91	90
mixing	SF96218	12/11/96	78	292	73	185	21.30	240	239	70	21.4	298	90	89
mix+clmp	SF96219	12/11/96	58	292	73	185	21.30	195	241	68	20.7	237	92	87
control	SF96220	12/12/96	99	296	72	185	19.80	277	240	69	20.3	380	88	89
clamped	SF96221	12/12/96	118	296	72	185	19.80	279	244	69	20.7	245	89	93
mix+clmp	SF96222	12/12/96	76	296	72	185	19.80	299	243	68	19.9	146	88	89

Appendix C, Table 13: Results with TBPS with Current Configuration Washing Single Units

			Pre Values (Taken after Thaw and Pr Waste Values							Goal >=70		Post Values (Taken after Processing) Goal <150	Goal >80%	Goal >80%	
Test #	Date of Test	Maximum Pressure (mmHg)	Weight of Blood In (g)	Hematocrit of Blood In (%)	Volume of RBCs In (ml)	Pre Sup. hgb (mg/dl)	Waste Volume (ml)	Waste Sup. hgb (mg/dl)	Post Weight of Blood (g)	Post Hematocrit	Post Total Hgb (g/dl)	Post Sup. hgb (mg/dl)	Recovery using Waste hgb (%)	Recovery using Pre / Post hgb (%)	Post Thaw Sup. Hb Adjusted Waste Hgb Recovery (%)
SF96224	12/19/96	194	669	21	128	1103	2128	562	281	66	19.6	171	80	78	88.30
SF96225	12/19/96	352	344	63	190	6524	2087	543	297	63	18.2	181	82	81	93.04
SF96226	12/19/96	81	279	73	177	919	2061	131	247	71	20.7	274	94	92	95.06
SF96227	12/20/96	-18	183	81	128	5006	1972	272	220	54	15.7	165	86	87	89.57
SF96228	12/20/96	69	288	76	189	3089	2067	355	239	69	20.0	355	85	88	89.14
SF96229	12/23/96	-6	232	79	158	2054	2037	172	222	67	19.8	261	92	90	93.69
SF96230	12/23/96	330	356	66	205	7289	2100	685	294	68	18.0	124	77	77	89.27
			335.9	65.6	167.8	3712.0	2064.6	388.6	257.1	65.4	18.9	218.7	85.2	84.7	91.2
			158.7	20.7	30.8	2585.9	50.2	212.0	33.1	5.6	1.7	80.6	6.2	6.2	2.7

Appendix D

Appendix D-1: Equipment and Disposable Descriptions

Appendix D-2: Disposable Description and Installation

Appendix D-3: Washing Instructions

Appendix D-1

EQUIPMENT AND DISPOSABLE DESCRIPTION

Console Description

The console is an electromechanical device for deglycerolized thawed frozen blood. It has a liquid tight upper surface which protects the internal components in the event of a liquid spill. It has a metal, polyurethane coated cabinet designed to withstand the effects of standard cleaning solutions (Figures 18 and 19).

Power Switch

The power switch is a standard rocker type located under the right front lip of the console.

Keypad

The keypad has twelve (12) key control buttons for the operation of the consol and is of the sealed membrane type and impervious to spills. The keypad surface facilitates easy cleaning.

Pressure Sensor

The pressure sensor monitors the blood filter's internal pressure.

Waste Bag Bracket

Mounting bracket for waste bag.

Magnetic Coupler

The magnetic coupler is a rotating device which will accept a properly inserted disposable filter. The magnetic coupler will gently rotate the internal disks housed inside the filter.

Hemoglobin and Glycerol Sensors

The hemoglobin sensor will monitor the amount of free hemoglobin in the waste line and the glycerol sensor will determine the glycerol concentration.

Pump

The pump is a two roller, occlusive pump, with a clear plastic protective cover, and a magnetic safety latch. (Cover MUST be closed in order to operate the system).

Storage Door

Hinged door for storage of keyboard.

Printer

The printer is a small stand-alone unit connected to the console by an RS 232 cable used for data reporting (output).

Keyboard

The keyboard is used for data entry with a key for each character in the full alphanumeric character set. The keyboard is of the sealed membrane type and is impervious to spills and facilitates easy cleaning.

IV Pole

A single telescoping IV pole for mounting reinfusion bag and heparinized saline bag.

Bar Code Reader

The bar code reader is a standard hand-held wand that is used for data entry with a reader of CODABAR and Code 128 format.

Pinch Valve

The pinch valve is used for controlling the direction of flow through the system.

Display

A sealed, display screen, continuously provides system status via a 4 line by 20 character, vacuum fluorescent test display. The display updates data every 1/2 second.

Shaker

The shaker attached to the console is a laboratory type shaker used to dilute the thawed blood prior to processing.

Appendix D-2

DISPOSABLE DESCRIPTION & INSTALLATION

The disposable filter set for the Thawed Blood Processing System (TBPS) is an exclusive set of disposable filters designed specifically for the TBPS Console as shown in Figure 16. The set incorporates the processing filter, reinfusion bag, waste bags, lines connecting to 12% and 0.9% saline bags, and a line to the thawed frozen blood.

Supplies Needed

- 12% saline bag
- 2 liter bag of 0.9% saline (processing solution)
- Thawed frozen blood
- Calibration tube

Note: The 12% and 0.9% saline must be warmed with the frozen blood at 37°C for about 20-30 minutes.

Installing the Disposable Filter Set

1. Raise the IV pole segments up until the red indicator mark is visible on each segment and tighten collar (Figure 20-A).
2. Remove the lid from the thawed blood processing system blood filter tray pack and set the tray by the console.
3. Lift the filter and tubing set from the tray and place the filter into the magnetic drive coupling of the console. Rotate the filter into position (Figure 20-B).
4. Remove the Waste Bag and hang it on the Waste Bag bracket on the right side of the console(Figure 20-C).
5. Open the pump cover and lift the crank handle. Position the tubing into the guide of the pump while rotating the pump counter-clockwise with the crank handle. Lower the crank handle back in place and close the pump cover. Attach cable locks to the tubing at the inlet side of the pump (Figure 20-D).
6. Install tubing per color code segment to the pinch valve from right to left (Orange to Green).
7. Firmly attach the mixing chamber on the Pinch Valve Panel with the Vent Port facing down.
8. Firmly attach the pressure line to the pressure meter (Figure 20-E).
9. Hang the thawed blood to be processed on the shaker and spike the bag with the orange spike.
10. Hang the 2 liter processing solution bag on the left hook of the pole on the console. With the clamp closed, spike the processing solution bag with the "White" color coded line (Figure 20-F).
11. Hang the 12% saline bag on the hook and, with clamp closed, spike the bag with the blue line spike.

WASHING INSTRUCTIONS

1. Verify that all connections are made and that all clamps are closed.
2. Insert calibration tube into the hemoglobin/glycerol sensor monitor and close and latch the cover (Figure 21-A).
3. Activate machine by making certain the TBPS is plugged in and turning the power switch to the "power on" position, as shown in Figure 21-B.
4. Open the Hemoglobin/glycerol Sensor Monitor Latch and remove the calibration tube
5. Insert waste line sensor tube into the Hemoglobin/Glycerol sensor Monitor and close and latch the cover (Figure 21-C).
6. User presses Auto on keypad
7. User presses Start on keypad

   Display:

   SCAN OR ENTER THE
   GLYCEROLIZED
   UNIT NUMBER:

   (FIELD DIMENSION = 10)
8. User enters glycerolized unit number via keyboard or barcode scanner.

   Display:

   RE-SCAN OR RE-ENTER
   THE GLYCEROLIZED
   UNIT NUMBER:

   (FIELD DIMENSION = 10)
9. User enters glycerolized unit number via keyboard or barcode scanner.
10. Are the numbers the same? If no beep for error go to 3. If yes, go to 8.
11. User enters deglycerolized unit number via keyboard or barcode scanner.

    Display:

    SCAN OR ENTER THE
    DEGLYCEROLIZED
    UNIT NUMBER:

    (FIELD DIMENSION = 10)
12. User enters deglycerolized unit number via keyboard or barcode scanner.

    Display:

    RE-SCAN OR RE-ENTER
    THE DEGLYCEROLIZED
    UNIT NUMBER:

    (FIELD DIMENSION = 10)
13. Are the numbers the same? If no beep for error, got to 8. If yes, got to 13.

Display:

SISTER GLYCEROLIZED
UNIT NUMBER:
(PRESS ENTER FOR NONE):

(FIELD DIMENSION = 10)

14. User enters sister glycerolized unit number via keyboard or barcode scanner or presses enter for none

Display:

SISTER GLYCEROLIZED
UNIT NUMBER VERIFY?
(PRESS ENTER FOR NONE):

(FIELD DIMENSION = 10)

15. User enters sister glycerolized unit number via keyboard or barcode scanner or presses enter for none
16. Are the numbers the same? If no beep for error, go to 13. If yes, go to 18.

Display:

SISTER GLYCEROLIZED
UNIT NUMBER VERIFY?
(PRESS ENTER FOR NONE):

(FIELD DIMENSION = 10)

17. User enters sister deglycerolized unit number via keyboard or barcode scanner or presses enter for none

Display:

SISTER GLYCEROLIZED
UNIT NUMBER VERIFY?
(PRESS ENTER FOR NONE):

(FIELD DIMENSION = 10)

18. User enters sister deglycerolized unit number via keyboard or barcode scanner or presses enter for none.
19. Are the numbers the same: If no beep for error, go to 18. If yes, go to 23.

Display:

SCAN OR ENTER THE
LOT NUMBER OF THE
12% SALINE: _____

(FIELD DIMENSION = 10)

20. User enters the lot number via keyboard or barcode scanner.

Display:

SCAN OR ENTER THE
LOT NUMBER OF THE
12% SALINE: _____

(FIELD DIMENSION = 10)

21. User enters the lot number via keyboard or barcode scanner.
22. Are the numbers the same? If no beep for error, go to 23. If yes, go to 28.

Display:

SCAN OR ENTER THE
LOT NUMBER OF THE
PROCESSING SOLUTION: _____

(FIELD DIMENSION = 10)

23. User enters the lot number via keyboard or barcode scanner.

Display:

RE-SCAN OR RE-ENTER
LOT NUMBER OF THE
PROCESSING SOLUTION: _____

(FIELD DIMENSION = 10)

24. User enters the lot number via keyboard or barcode scanner.
25. Are the numbers the same: If no beep for error, go to 28. If yes, go to 33.]

Display:

SCAN OR ENTER THE
LOT NUMBER OF THE
ADDITIVE: _____

(FIELD DIMENSION = 10)

26. User enters the name via keyboard

Display:

ENTER THE NAME
OF THE
ADDITIVE: _____

(FIELD DIMENSION = 10)

27. User enters the name via keyboard.

Display:

RE-SCAN OR RE-ENTER THE
THE LOT NUMBER OF THE
ADDITIVE: _____

(FIELD DIMENSION = 10)

28. User enters the lot number via keyboard or barcode scanner.
29. Are the numbers the same? If no beep for error, go to 35. If yes, go to 40.
30. User presses "Start" on keypad and simultaneously opens the 12% saline line clamp, processing solution line clamp; thawed blood line clamp at shaker, product bag line clamp and waste line clamp.
31. When the processing solution bag is empty, the machine automatically stops. Immediately and simultaneously open the clamp on the product line to the blood bag and clamp off the waste line.
32. Remove the cap from the top vent line on filter and connect a syringe to the vent line. Use the syringe to expel the blood from the filter by injecting air into the top vent. Additional blood may be collected by lifting up filter form the magnetic drive coupling of the console and turning the filter on its side.
33. After all sequences are completed, printer automatically prints out report in the attached format.

34. Turn the power switch to the "Power off" position.

35. Discard all disposables in accordance with lab biohazard regulations and procedures.

Printed Report

EXPIRATION DATE AND TIME: MM/DD/YY HH:MM GMT

DEGLYCEROLIZED UNIT NUMBER: _____

HEMATOCRIT OF DEGLYCEROLIZED UNIT: _____%

PLASMA HEMOGLOBIN OF DEGLYCEROLIZED UNIT: _____mg/dl

GLYCEROL LEVEL OF DEGLYCEROLIZED UNIT: _____%

START TIME OF WASH PROCESS: MM/DD/YY HH:MM GMT

STOP TIME OF WASH PROCESS: MM/DD/YY HH:MM GMT

LOT NUMBER OF 12% SALINE: _____

LOT NUMBER OF PROCESSING SOLUTION: _____

ADDITIVE NAME: _____

LOT NUMBER OF ADDITIVE: _____

GLYCEROLIZED UNIT NUMBER: _____

SISTER DEGLYCEROLIZED UNIT NUMBER: _____

SISTER GLYCEROLIZED UNIT NUMBER: _____

CONSOLE SERIAL NUMBER: _____

ADVANCED HAEMOTECHNOLOGIES
2828 N. CRESCENT RIDGE DR.
THE WOODLANDS, TX 77381
TEL: 800.669.9001
FAX: 281.292.7930

Caution: Actual performance results of this device may vary depending on many in-use variables. These may include the condition of frozen thawed blood, hemolysis, etc.

Warning: Safe and effective use of TBPS devices requires application of proper techniques of setup and operation according to AHT instructions. Accordingly, the set-up and processing of fluids or blood in this equipment should be undertaken by trained personnel only.

Appendix E

E-1: Software Requirements Specification

E-2: Software Validation Plan

E-3: Software Validation Report

Software Requirements Specification

for the
ADVANCED HAEMOTECHNOLOGIES
Thawed Blood Processing System

SECTION CONTENTS PAGE

1.0 Introduction

1.1 Purpose

This document comprises the Software Requirements Specification (SRS) for the development of the software to be used in the Thawed Blood Processing System (TBPS) manufactured by Surgimedics - Advanced Haemotechnologies (AHT). The purpose of this specification is to describe the context of the software in terms of its interface with the system hardware and the functionality of the software in terms of its response to inputs from the hardware. The following questions will be answered:

1. What is the software supposed to do?

2. How does the software interact with people, the system's hardware, other hardware, and other software?

3. What is the speed, availability, response, time, recovery time of various software functions, etc.?

4. What are the portability, correctness, maintainability, security, etc. considerations?

5. Are there any required standards in effect, implementation language, policies for database integrity, resource limits, operating environments, etc.?

The SRS does not contain any design or project requirements. Those are included in the Software Design Description (SDD). The SRS document contains information which AHT considers proprietary and is not to be disseminated without prior written consent from AHT.

1.2 Scope

The software will be running on the TBPS console used to deglycerolize thawed frozen blood. The software will allow for user entry into a database, process control, and report printing. The software will not allow for process deviation, database transfer, and user customization.

The objective of the software is to control the display, operator keypad, timers, sensors, filter motor, pump motor, pinch valves, speaker, barcode scanner, power control board, printer, and keyboard. The benefit is a totally automated sequence of washing thawed frozen blood. The goal of the software is to therefore have a system that is capable of controlling all the inputs and outputs while maintaining safety in all situations.

1.3 Definitions, Acronyms, and Abbreviations

The user is the person or persons that operate or interact directly with the product. The developer is the person or persons developing the software product. The TBPS is the Thawed Blood Processing System, that is the final product. The SRS is the Software Requirements Specification and the SDD is the Software Design Descriptions. The abbreviation std. refers to standard and the abbreviation spec. refers to specification. A/D refers to both analog to digital and digital to analog while CPU stands for the central processing unit. GMP stands for the United States Food and Drug Administration's (FDA) good manufacturing practices guidelines. ISO stands for the International Safety Organization and AHT MIS refers to Surgimedics - Advanced Haemotechnologies' management information systems division.

1.4 References

The following documents are referenced in other sections of the SRS:

1. IEEE std. 830-1993, IEEE Inc. 1994.

2. IEEE std. 1016-1987, IEEE Inc., 1994.

1.5 Overview

The SRS contains a table of contents, introduction, overall description, specific requirements, appendices, and index.

The first section of this document (1.0 Introduction) provides an overview of the entire SRS. Subsections are included for the purpose and scope of the SRS, as well as definitions, references and this overview.

Section 2 describes the general factors that affect the product and its requirements. This section does not state specific requirements; instead, it provides a background for those requirements, which are defined in detail in section 3 and makes them easier to understand.

Section 3 contains all the software requirements to a level of detail sufficient to enable designers to design a system to satisfy those requirements, and testers to test that the system satisfies those requirements. Throughout section 3, every stated requirement includes a description of every input, every output, and all functions performed by the system in response to an input or in support of an output.

2.0 Overall Description

2.1 Product Perspective

The TBPS software will be used to exclusively run the TBPS console for deglycerolizing thawed frozen blood. Other related products are all hardware, consisting of; laboratory shakers, sterile docking systems, heat sealers, centrifuges, heated water baths, and freezers.

The TBPS software will be accessible through MS-DOS (Windows is not a requirement). The user interfaces with the software through a display, keypad, keyboard, barcode scanner, speaker, and a printer. The following lists the details for each interface:

1. Display - 4 line by 20 character vacuum fluorescent display, connected via the printer port
2. Keypad - 12 button keypad that allows for control of user functions, connected to the digital input/output of the processor
3. Keyboard - 101 keyboard or equivalent for data entry, connected via the keyboard port
4. Barcode scanner - wand type with keyboard wedge to connect via the keyboard port
5. Speaker - 5 Volt type connected via the digital input/output of the processor
6. Printer - thermal printer connected via the 9-pin serial port

The hardware is a 386 based processor that interfaces with a display, keypad, keyboard, barcode scanner, speaker, printer, opto-isolation board, A/D board, interface board, servo motor controllers, and power control board. The 386 requires 4 megabytes of RAM. The software itself interfaces with a "C" compiler and a database.

2.2 Product Functions

The primary function of the software will be to control the process of deglycerolizing thawed frozen blood. The major functions that the software will perform are as follows:

1. Control the TBPS
2. Perform system initialization functions
   - 2.1. Perform control system initialization functions
   - 2.2. Manage hardware initialization and CPU/memory diagnostics
3. Perform calibration and normal operation functions
   - 3.1. Perform calibration functions
   - 3.2 Perform normal operations
4. Perform abnormal operation functions

2.3 User Characteristics

Users should be familiar with the processing of thawed frozen blood. Users should have a knowledge of blood handling procedures. Typical users of this product would include laboratory technicians, blood bank technicians, and other personnel involved in the processing of blood.

2.4 Constraints

The software shall meet the following constraints:

1. The program shall meet all GMP, Proposed/Revised GMP, and ISO requirements for software validation.
2. The program shall be compatible with computer and network systems accepting MS-DOS version 6.0 or higher.
3. The program shall interface and be compatible with a "C" compiler and a database.
4. The program shall comply with all standard AHT MIS software design standards and conventions.

2.5 Assumptions and Dependencies

The program shall be developed under the assumption that users will have a basic knowledge of blood bank equipment and blood bank regulations as related to the deglycerolizing of thawed frozen blood and the reinfusion of such blood.

3.0 Specific Requirements

3.1 External Interfaces

User input will consist of keystrokes on the keyboard and keypad and scanned digits from the barcode scanner. Other inputs to the software will be from the sensor inputs on the digital input/output and A/D board.

User outputs will consist of a display, speaker, and printer. Other outputs from the software will be to sensors and drivers through the digital input/output board and A/D board.

3.2 Functions

3.2.1 Control the TBPS

The following diagrams (Figure 3.2.1.1 and Figure 3.2.1.2) represent the context of the control functions of the TBPS. The TBPS software interfaces with all the items in Figure 3.2.1.1. The

TBPS software also controls the operation of the system by performing the sets of functions laid out in Figure 3.2.1.2.

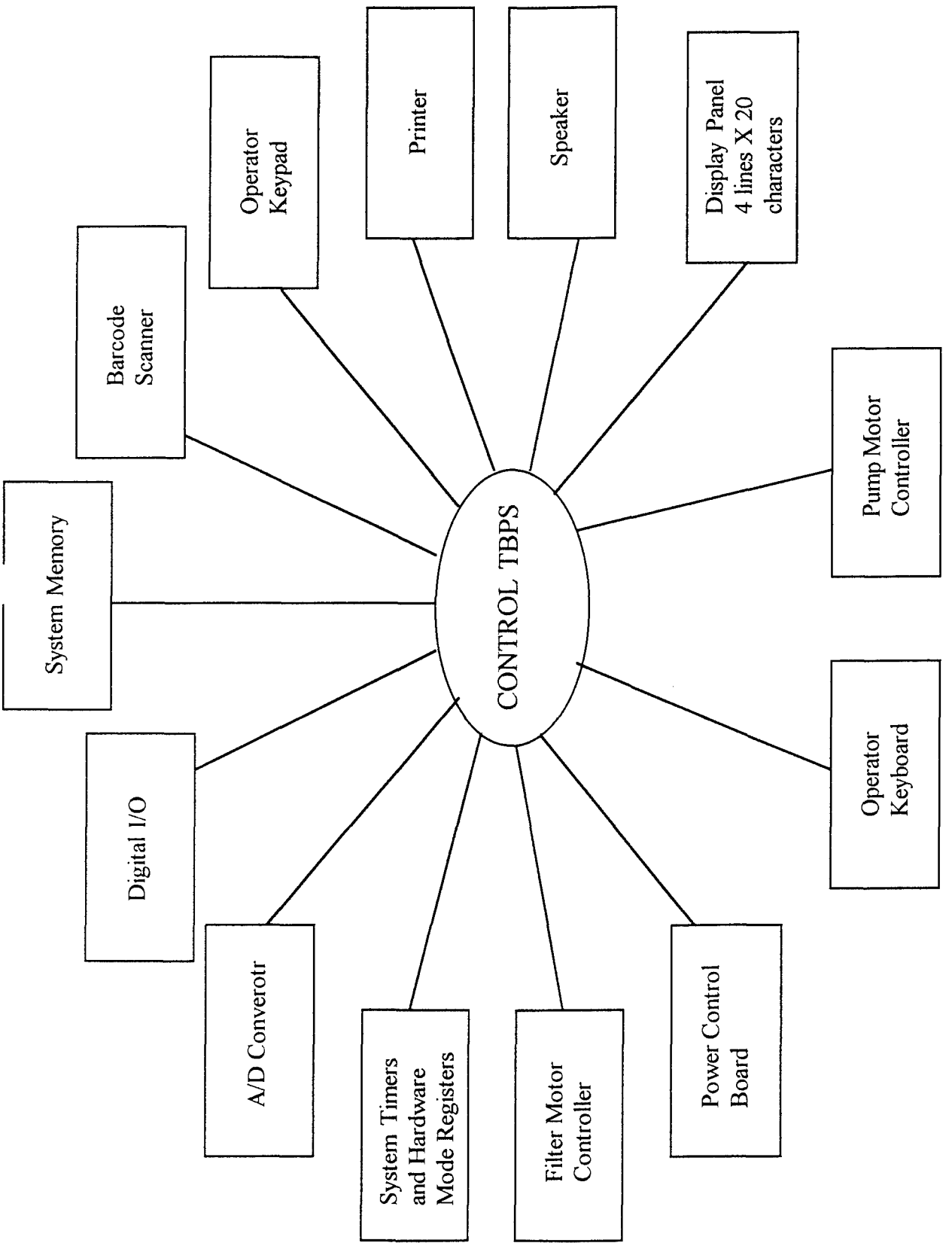


Figure 3.2.1.1 Context Diagram

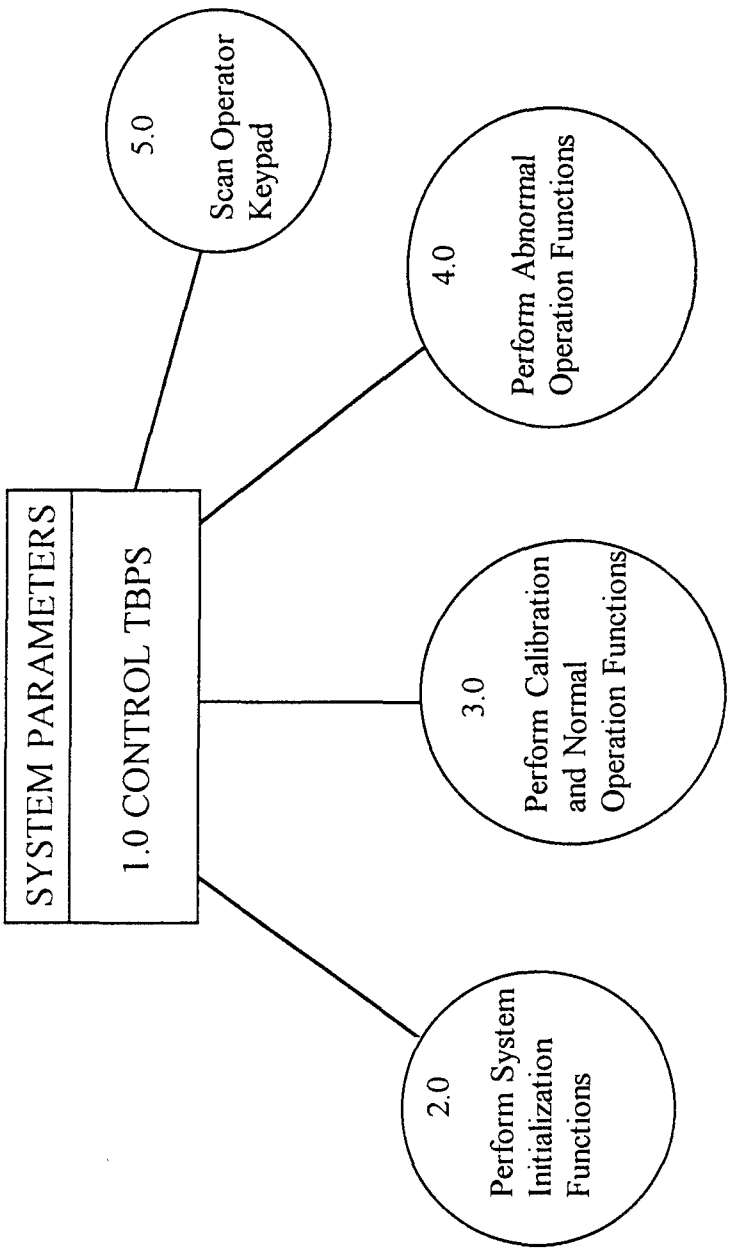


Figure 3.2.1.2 Control TBPS

3.2.2 Perform System Initialization Functions

The TBPS software must initialize the hardware and software functions in order to operate. In order to initialize the hardware and software; the hardware initialization and CPU and memory diagnostics must be managed, the power control diagnostics must be performed, and the A/D diagnostics must be performed. The following figures, Figure 3.2.2.1 and Figure 3.2.2.2, show the general configuration of the system initialization.

The CPU and memory diagnostics, the first step in initializing the hardware and software, are done by performing a RAM memory test, a ROM checksum test, a CPU test, and validating system parameters. The following figures, Figure 3.2.2.3 and Figure 3.2.2.4, represent the configuration of the CPU and memory diagnostics.

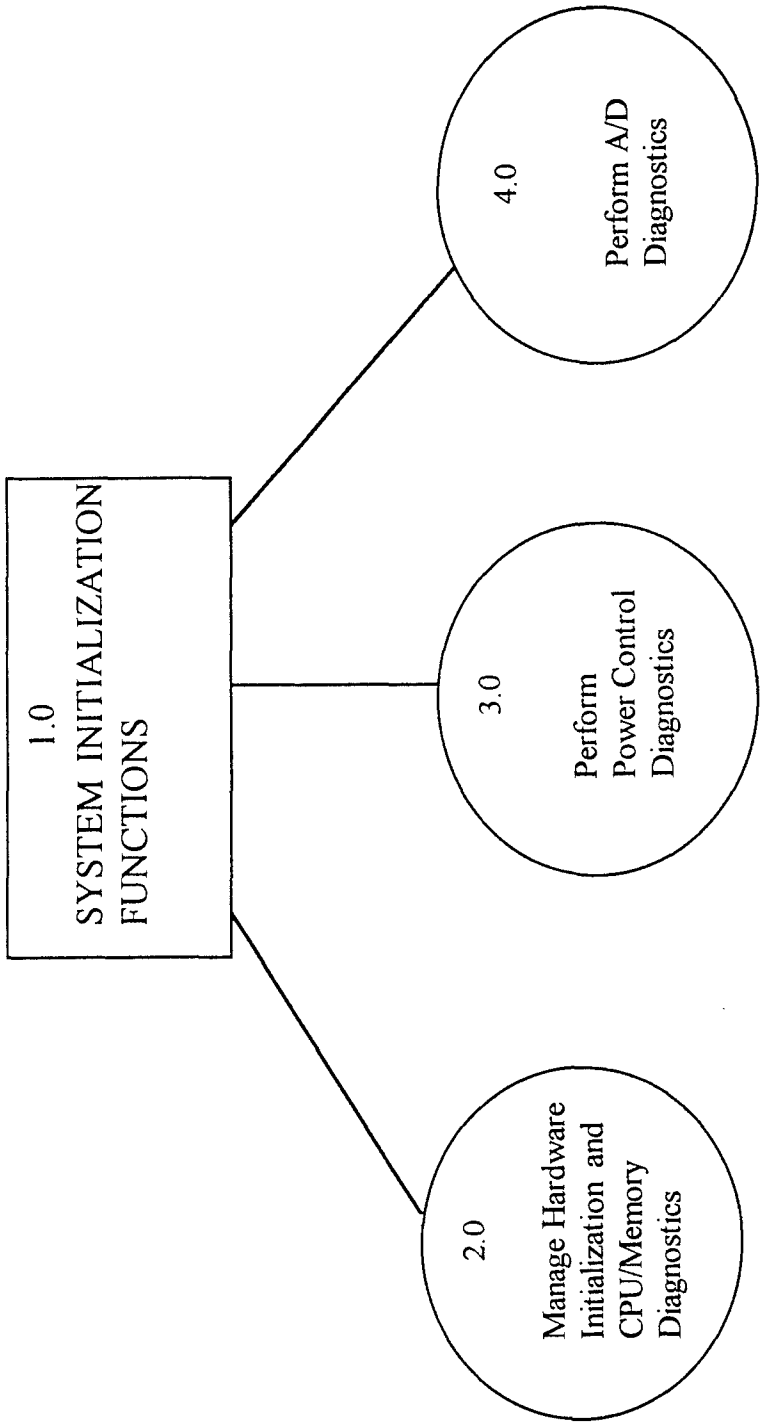


Figure 3.2.2.1 System Initialization Functions

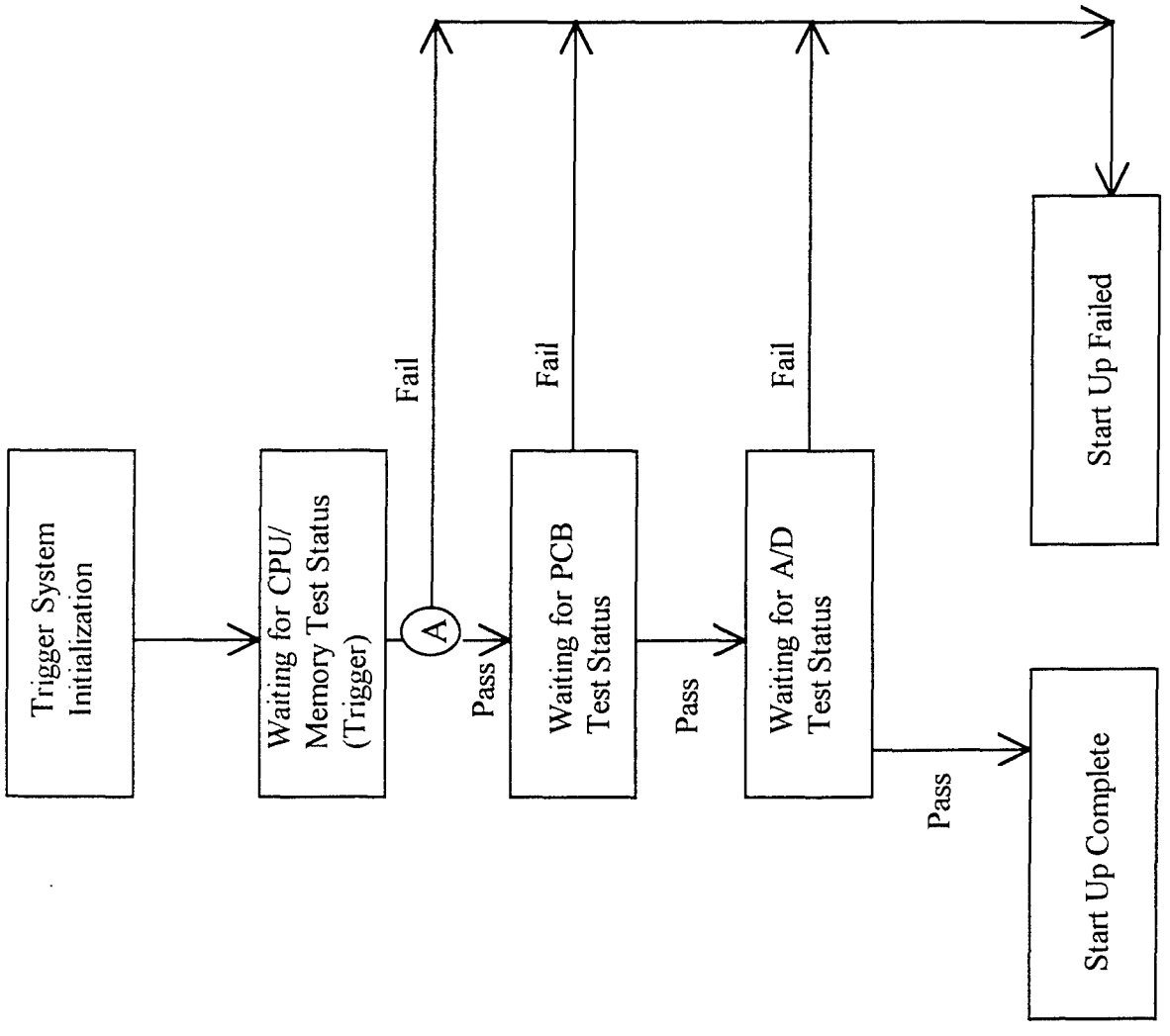


Figure 3.2.2.2 Flowcharrt of System Initialization Functions

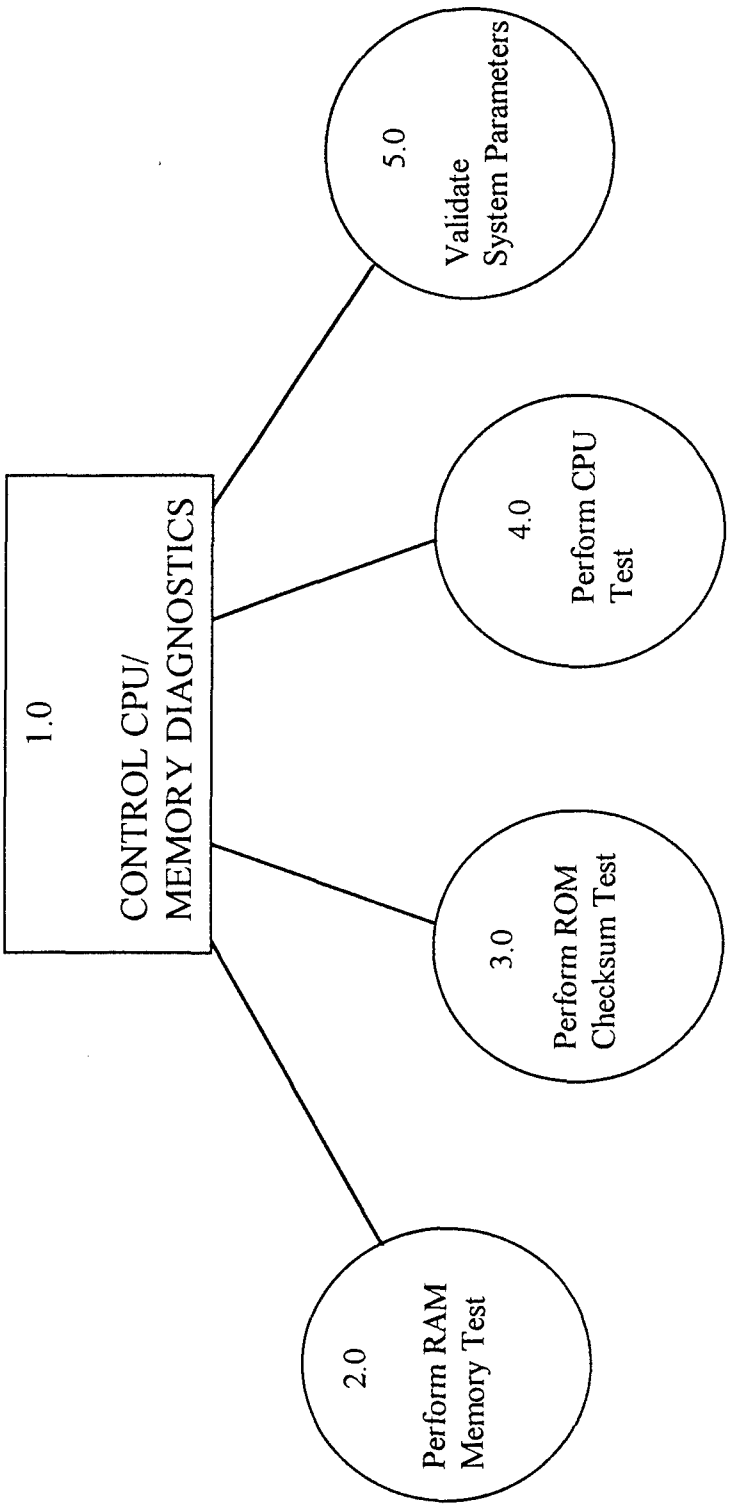


Figure 3.2.2.3 CPU and Memory Diagnostics

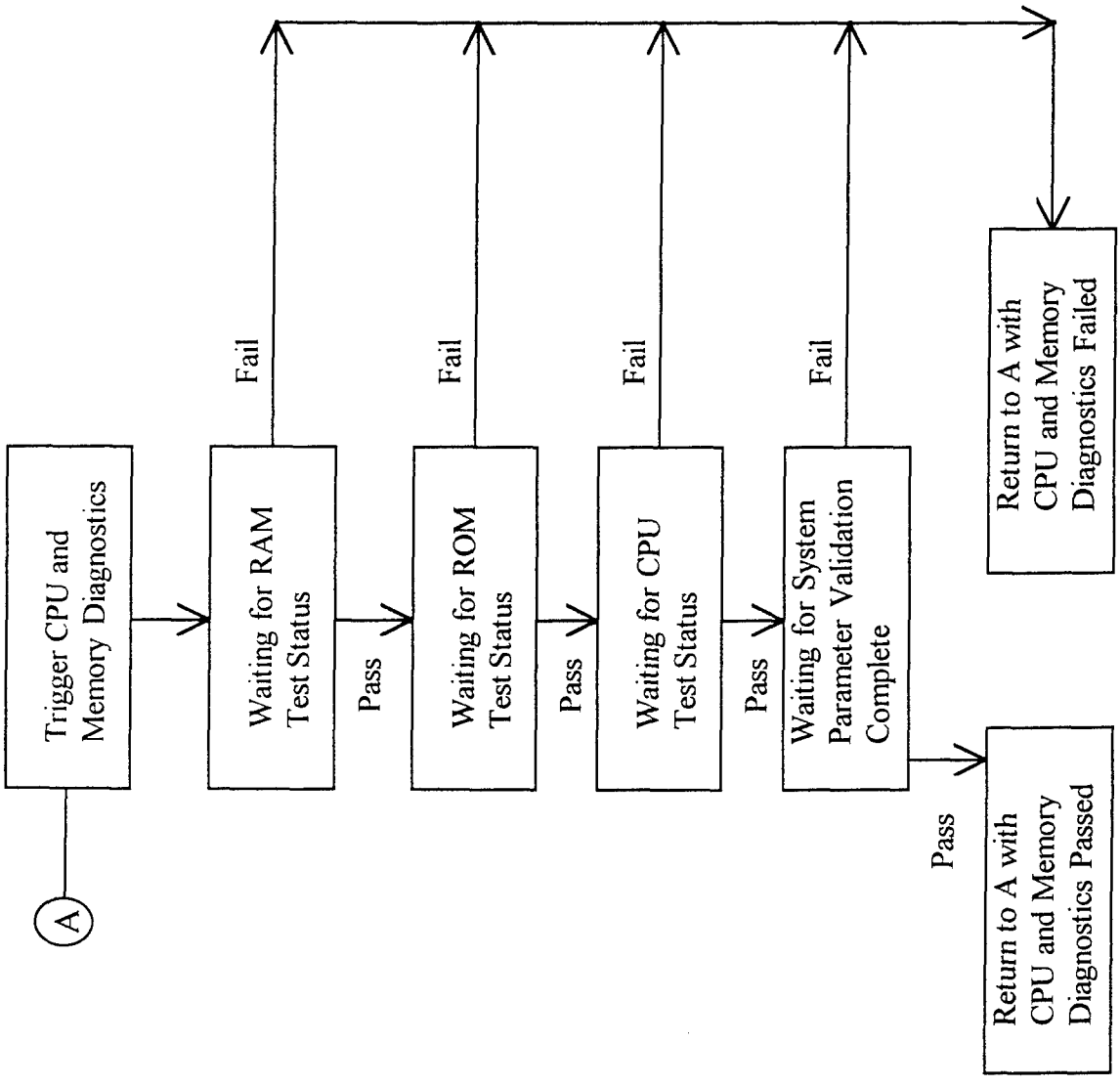


Figure 3.2.2.4 Flowchart of CPU and Memory Diagnostics

3.2.3 Perform Calibration and Normal Operation Functions

The TBPS software must perform calibration and normal operation functions as illustrated in Figure 3.2.3.1. The calibration functions must calibrate the motors, pressure transducer, and other sensors. Figure 3.2.3.2 illustrates the calibration functions.

The normal operation functions must control the manual functions and automated functions (Figure 3.2.3.3). The manual functions allow for the control of the display, pump motor, rotor motor, and controlled outputs to the pinch valves, compressor, and external shaker (Figure 3.2.3.4). The rotor motor speed is adjustable along with the pump motor. The pinch valves may be turned on or off along with the external shaker and compressor. The display is running at all times displaying the rotor speed, pump speed, load on the rotor motor, and which controlled outputs are on and which ones are off. The keypad is used to input the user controlled manual changes in rotor speed, motor speed, pinch valve closure, compressor on/off, and shaker on/off. The manual functions also get real time data and real time alarms for displaying the parameters.

The automated functions control the pump motor, rotor motor, digital I/O, A/D board, printer, display, barcode scanner, speaker, keyboard, and clock. The automated function will get real time data, get real time alarms, get the automated sequence, perform the automated sequence, and interpret clock data (Figure 3.2.3.5). The automated function will get the sequence to be used to run the console by the user pressing the "Auto" button on the keypad. After retrieval of the sequence, the automated sequence will be performed by the user pressing the "Start" button on the keypad. The automated sequence will be performed by prompting and getting user entered data, running the sequence, and printing a report (Figure 3.2.3.6). Appendix A contains the format for prompting and getting user entered data. Running the automated sequence will control the pump motor, rotor motor, printer, display, barcode scanner, speaker, compressor, pinch valves, keyboard, and external shaker while getting real time data, real time alarms, and clock data (Figure 3.2.3.7). Appendix B contains the format for the printed report at the completion of the automated sequence.

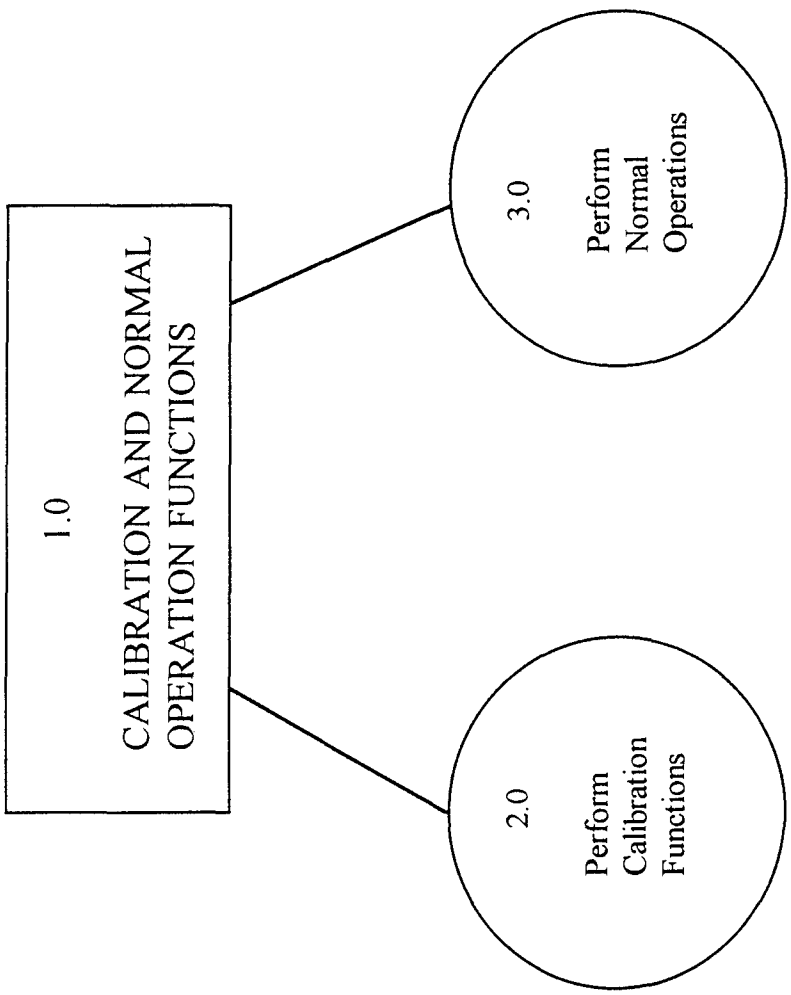


Figure 3.2.3.1 Calibration and Normal Operation Functions

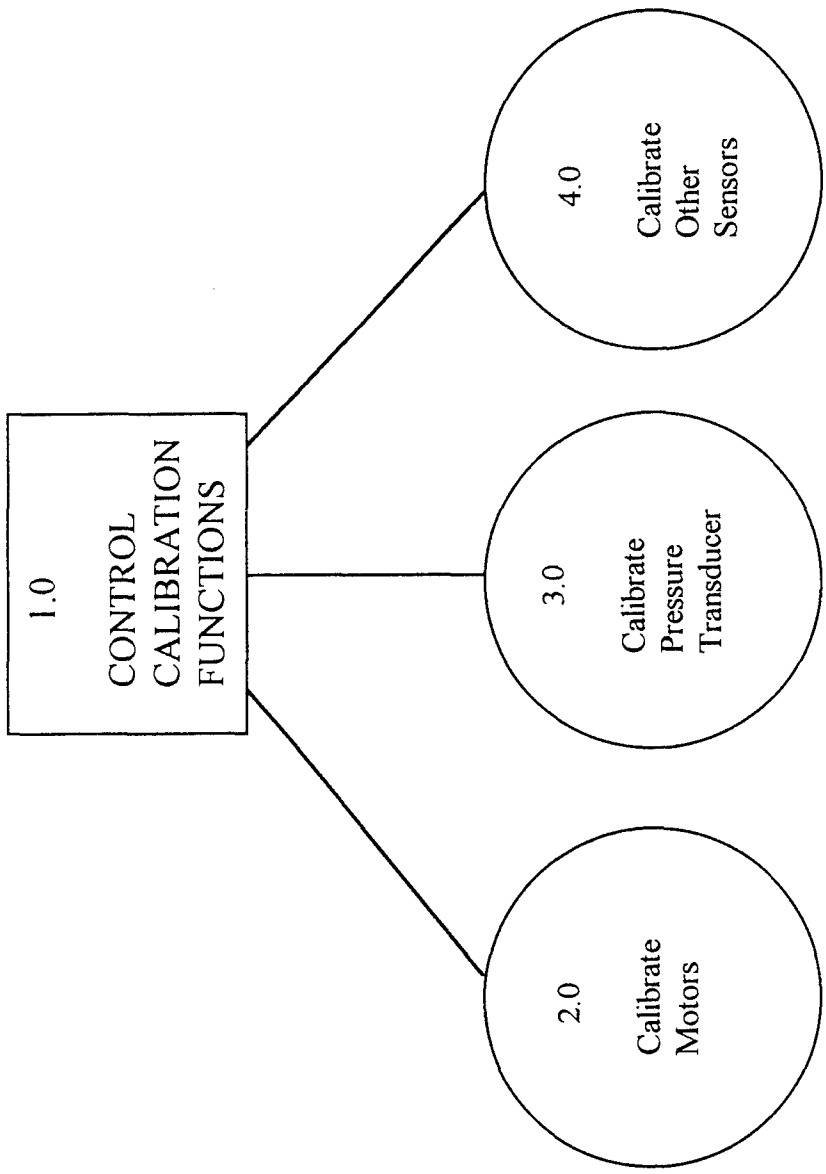


Figure 3.2.3.2 Calibration Functions

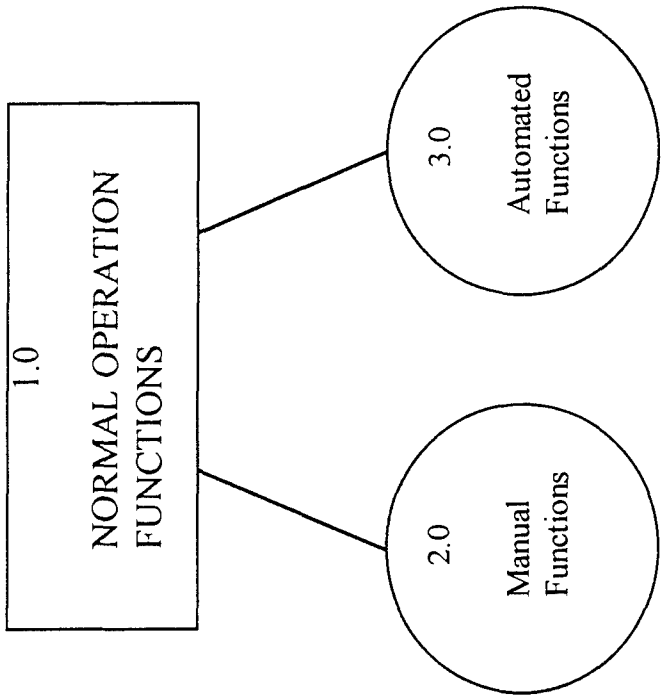


Figure 3.2.3.3 Normal Operation Functions

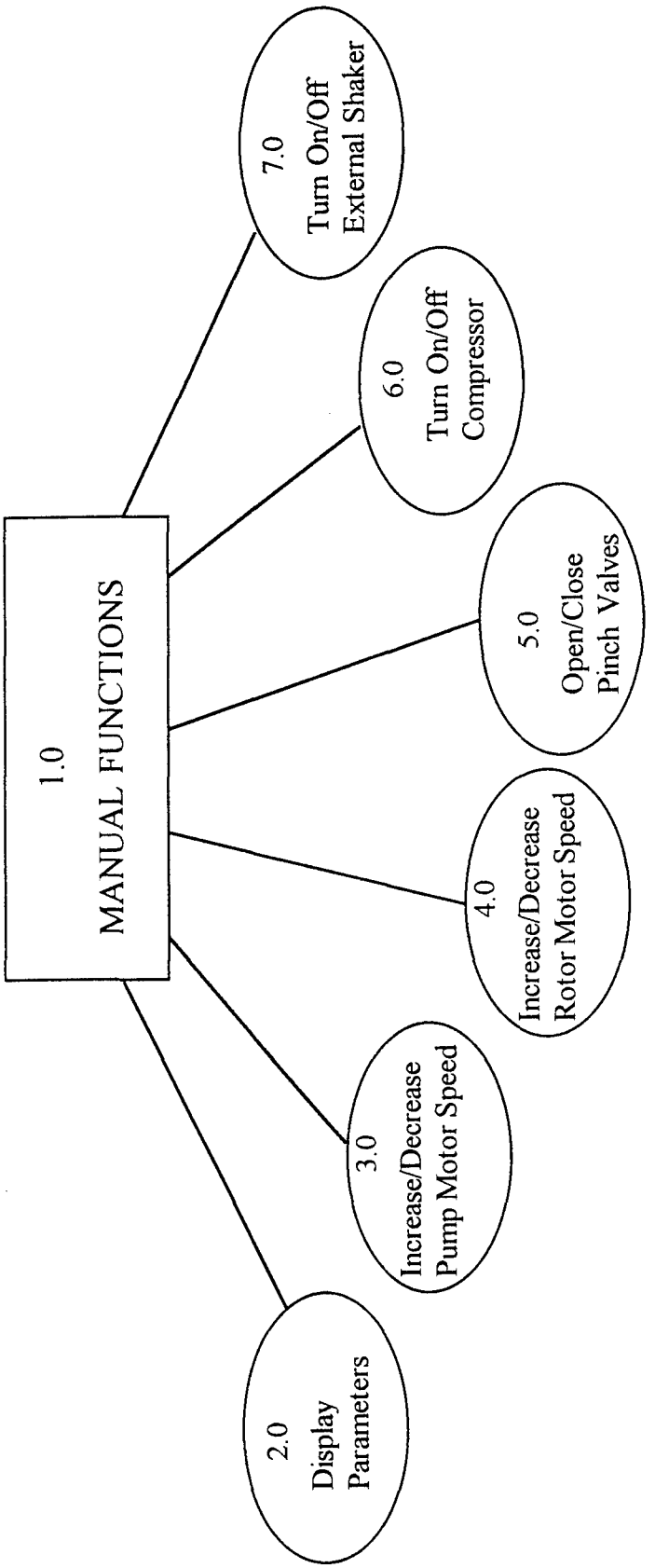


Figure 3.2.3.4 Manual Functions

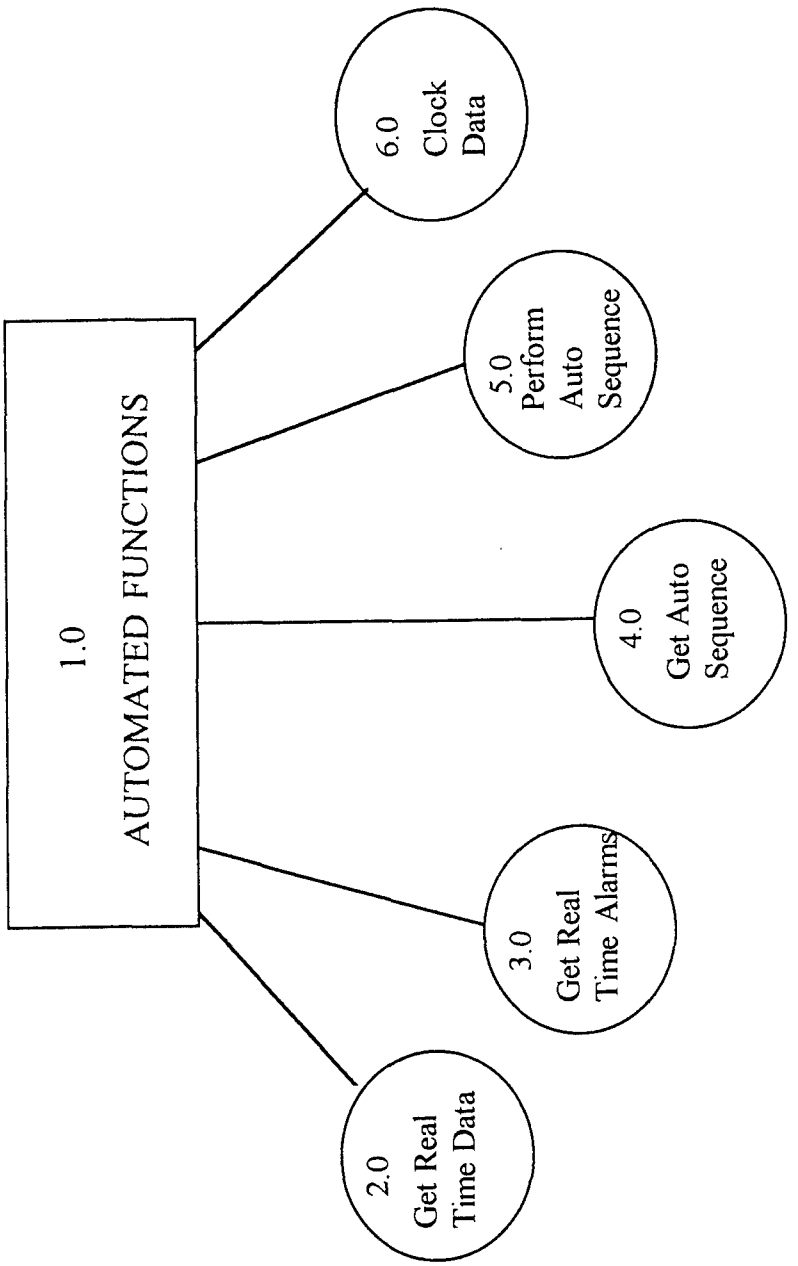


Figure 3.2.3.5 Automated Functions

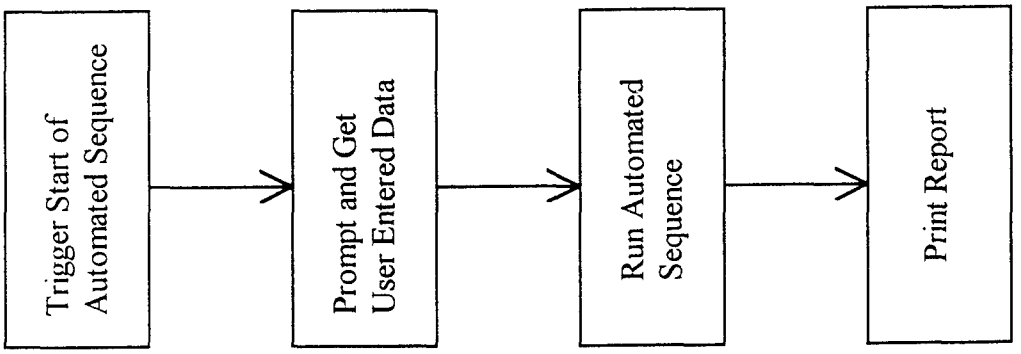


Figure 3.2.3.6 Perform Auto Sequence

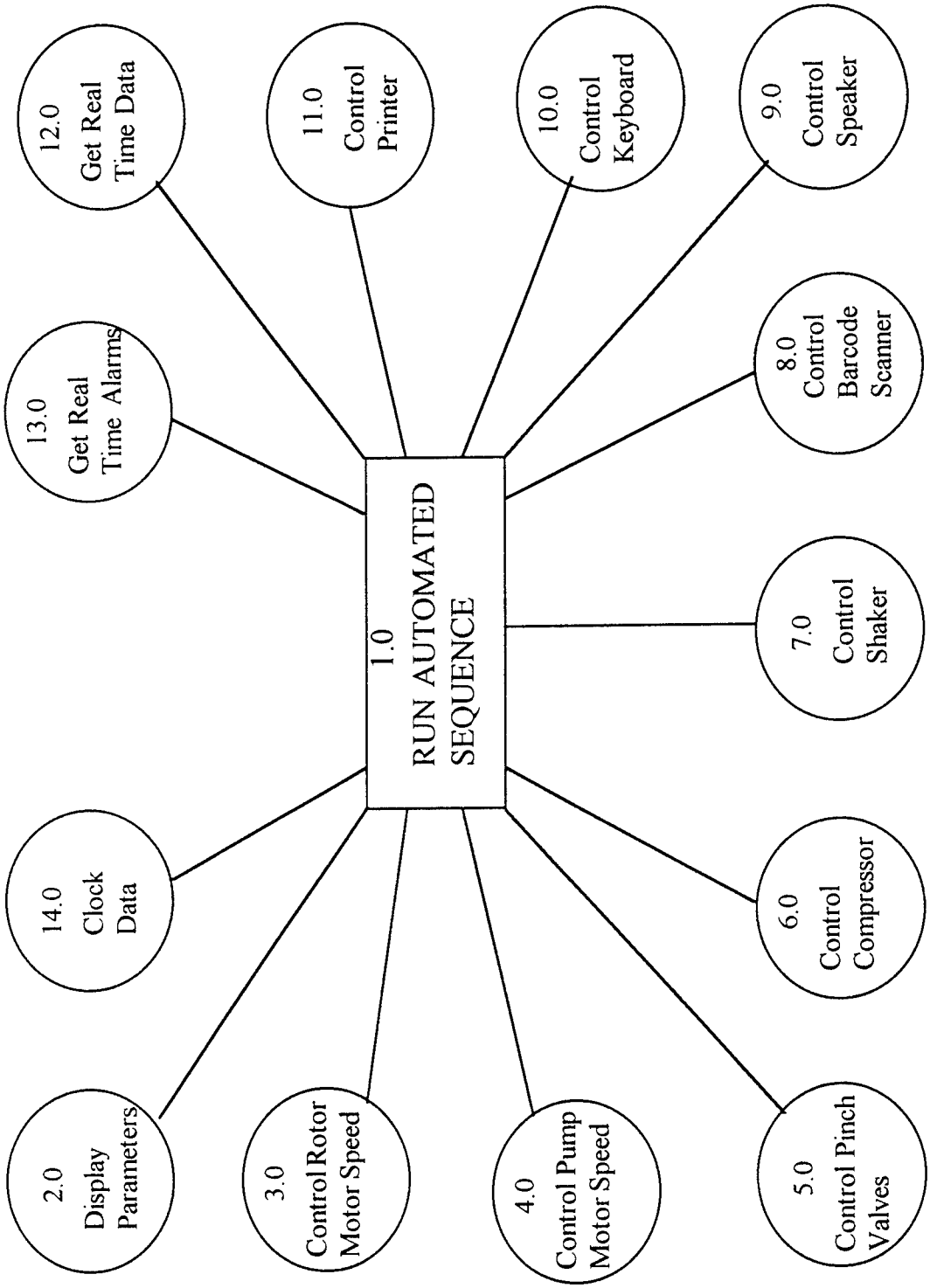


Figure 3.2.3.7 Run Automated Sequence

3.2.4 Perform Abnormal Operation Functions

In the case of an alarm or abnormal operation the software must respond. Therefore, the step of getting real time alarms is done during the automated sequence and manual controls. The alarms are separated here to stress the importance of their function. Figure 3.2.4.1 illustrates the process of getting real time alarms. If any one of the alarms is tripped, the software will notify the user on the speaker, display, and printer and will shut down the operation. Appendix C contains the user notification screens and Appendix D contains the error notification report that will be displayed and printed in case of an alarm.

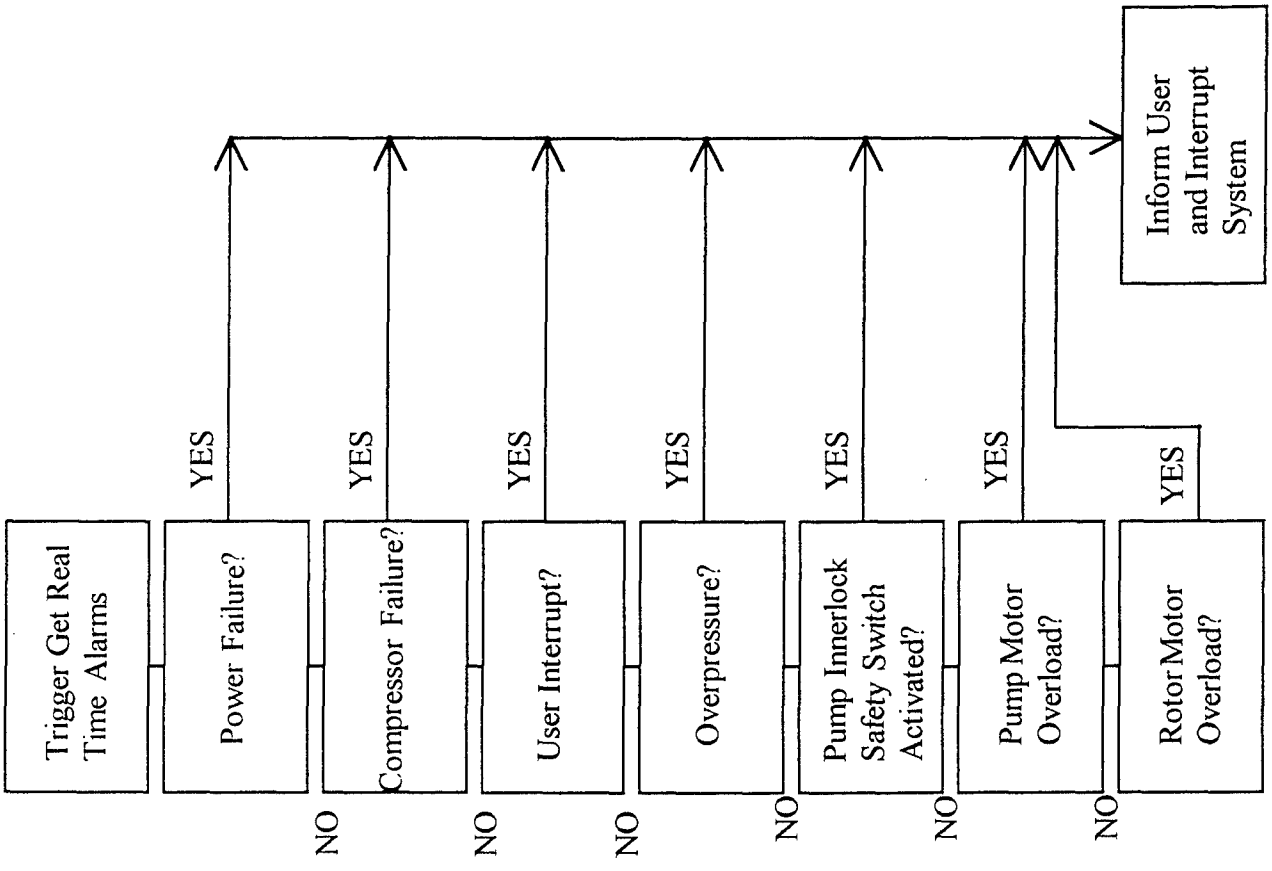


Figure 3.2.4.1 Get Real Time Alarms

3.3 Performance Requirements

The software should load and run on a 4 megabyte 386 processor within a reasonable amount of time. The software should be able to load the automated sequence in less than 5 seconds.

3.4 Logical Database Requirements

The database should be designed in D-base and should use GMP standards.

3.5 Design Constraints

The system must run on IBM compatible PC's 386 SX or better on the TBPS console.

3.6 Software System Attributes

3.6.1 Reliability

The system should be able to handle power surges without memory loss.

3.6.2 Availability

The system is only available on the TBPS console.

3.6.3 Security

Security is not required for the software.

3.6.4 Maintainability

The system is self maintained and if new versions are released, upgrades will be possible. If the system fails to initialize the hardware and software, fails calibration, or normal operations fail, the software will shut down the system and notify the user of the error. The user manual will direct the user to respond to the error messages.

3.6.5 Portability

The system is not portable.

Appendix A Prompt and Get User Entered Data

1. USER PRESSES AUTO ON KEYPAD
2. USER PRESSES START ON KEYPAD

3. DISPLAY:

   SCAN OR ENTER THE
   GLYCEROLIZED
   UNIT NUMBER:
   _____ (FIELD DIMENSION = 10)

4. USER ENTERS GLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER
5. DISPLAY:

   RE-SCAN OR RE-ENTER
   THE GLYCEROLIZED
   UNIT NUMBER:
   _____ (FIELD DIMENSION = 10)

6. USER ENTERS GLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER

7. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 3
   IF YES GO TO 8

8. DISPLAY:

   SCAN OR ENTER THE
   DEGLYCEROLIZED
   UNIT NUMBER:
   _____ (FIELD DIMENSION = 10)

9. USER ENTERS DEGLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER
10. DISPLAY:

    RE-SCAN OR RE-ENTER
    THE DEGLYCEROLIZED
    UNIT NUMBER:
    _____ (FIELD DIMENSION = 10)

11. USER ENTERS DEGLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER

12. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 8
IF YES GO TO 13

13. DISPLAY:

SISTER GLYCEROLIZED
UNIT NUMBER?
(PRESS ENTER FOR
NONE): _____ (FIELD DIMENSION = 10)

14. USER ENTERS SISTER GLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER OR PRESSES ENTER FOR NONE
15. DISPLAY:

SISTER GLYCEROLIZED
UNIT NUMBER VERIFY?
(PRESS ENTER FOR
NONE): _____ (FIELD DIMENSION = 10)

16. USER ENTERS SISTER GLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER OR PRESSES ENTER FOR NONE

17. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 13
IF YES GO TO 18

18. DISPLAY:

SISTER DEGLYC.
UNIT NUMBER?
(PRESS ENTER FOR
NONE): _____ (FIELD DIMENSION = 10)

19. USER ENTERS SISTER DEGLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER OR PRESSES ENTER FOR NONE
20. DISPLAY:

SISTER DEGLYC.
UNIT NUMBER VERIFY?
(PRESS ENTER FOR
NONE): _____ (FIELD DIMENSION = 10)

21. USER ENTERS SISTER DEGLYCEROLIZED UNIT NUMBER VIA KEYBOARD OR BARCODE SCANNER OR PRESSES ENTER FOR NONE

22. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 18
IF YES GO TO 23

23. DISPLAY:

SCAN OR ENTER THE
LOT NUMBER OF THE
12% SALINE:
_____ (FIELD DIMENSION = 10)

24. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER
25. DISPLAY:

RE-SCAN OR RE-ENTER
THE LOT NUMBER
OF THE 12% SALINE:
_____ (FIELD DIMENSION = 10)

26. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER

27. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 23
IF YES GO TO 28

28. DISPLAY:

SCAN OR ENTER THE
LOT NUMBER OF THE
PROCESSING
SOLUTION:_____ (FIELD DIMENSION = 10)

29. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER
30. DISPLAY:

RE-SCAN OR RE-ENTER
THE LOT NUMBER OF
THE PROCESSING
SOLUTION:_____ (FIELD DIMENSION = 10)

31. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER

32. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 28
IF YES GO TO 33

33. DISPLAY:

ENTER THE NAME
OF THE ADDITIVE:
_____ (FIELD DIMENSION = 20)

34. USER ENTERS THE NAME VIA KEYBOARD

35. DISPLAY:

SCAN OR ENTER THE
LOT NUMBER OF THE
ADDITIVE:
_____ (FIELD DIMENSION = 10)

36. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER
37. DISPLAY:

RE-SCAN OR RE-ENTER
THE LOT NUMBER
OF THE ADDITIVE:
_____ (FIELD DIMENSION = 10)

38. USER ENTERS THE LOT NUMBER VIA KEYBOARD OR BARCODE SCANNER

39. ARE THE NUMBERS THE SAME? IF NO BEEP FOR ERROR AND GO TO 35
IF YES GO TO 40

40. RUN THE AUTOMATED SEQUENCE IN TBPS.SEQ
41. AFTER SEQUENCE IS RUN PRINT OUT REPORT IN ATTACHED FORMAT

Appendix B Printed Report

EXPIRATION DATE AND TIME: MM/DD/YY HH:MM

DEGLYCEROLIZED UNIT NUMBER: _____

FINAL HEMOGLOBIN OF WASTE: _____mg/dl

GLYC. LEVEL OF DEGLYC. UNIT: _____%

START WASH PROCESS: MM/DD/YY HH:MM

STOP WASH PROCESS: MM/DD/YY HH:MM

LOT NUMBER OF 12% SALINE: _____

LOT NUMBER OF PROC. SOLUTION: _____

ADDITIVE NAME: _____

LOT NUMBER OD ADDITIVE: _____

GLYCEROLIZED UNIT NUMBER: _____

SISTER DEGLYC. UNIT NUMBER: _____

SISTER GLYC. UNIT NUMBER: _____

CONSOLE SERIAL NUMBER: _____

ADVANCED HAEMOTECHNOLOGIES
2828 N. CRESCENT RIDGE DR.
THE WOODLANDS, TEXAS, USA 77381
TEL: 800.669.9001
FAX: 281.292.7930

Appendix C User Notification Screens for Errors

In case of power failure the screen will display:

POWER FAILURE
DISPOSE OF UNIT
PRESS STOP TO
START NEW UNIT

In case of over pressure (the filter pressure exceeds 500 millimeters of Mercury) the screen will display:

OVER PRESSURE
DISPOSE OF UNIT
PRESS STOP TO
START NEW UNIT

In case of the pump innerlock safety switch activating the screen will display:

PUMP OPEN
DISPOSE OF UNIT
PRESS STOP TO
START NEW UNIT

In case of the rotor motor being overloaded the screen will display:

MOTOR OVERLOAD
DISPOSE OF UNIT
PRESS STOP TO
START NEW UNIT

Appendix D Printed Error and Disposal Report

GLYCEROLIZED UNIT NUMBER: _____

DEGLYCEROLIZED UNIT NUMBER: _____

ERROR AND DISPOSAL OF UNIT DUE TO:
_____

CONSOLE SERIAL NUMBER: _____

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Software Validation Plan

for the
ADVANCED HAEMOTECHNOLOGIES
Thawed Blood Processing System

1.0 Purpose

This document comprises the Software Validation Plan (SVP) for the development of the software to be used in the Thawed Blood Processing System (TBPS) manufactured by Surgimedics - Advanced Haemotechnologies (AHT). The purpose of this plan is to describe the protocol used to validate the correct function of the software. The following items will be tested:

1. System initialization functions.
2. Calibration functions.
3. Normal operation functions in automatic and manual mode.
4. Abnormal operation functions in automatic mode.

The SVP does not contain any design or project requirements. Those are included in the Software Design Description (SDD). The SVP document contains information which AHT considers proprietary and is not to be disseminated without prior written consent from AHT.

2.0 Referenced Documents

The following documents are referenced in other sections of the SVP:

1. IEEE std. 830-1993, IEEE Inc. 1994.

2. IEEE std. 1016-1987, IEEE Inc., 1994.

3.0 Definitions

The user is the person or persons that operate or interact directly with the product. The developer is the person or persons developing the software product. The TBPS is the Thawed Blood Processing System, that is the final product. The SRS is the Software Requirements Specification and the SDD is the Software Design Descriptions. The abbreviation std. refers to standard and the abbreviation spec. refers to specification. A/D refers to both analog to digital and digital to analog while CPU stands for the central processing unit. GMP stands for the United States Food and Drug Administration's (FDA) good manufacturing practices guidelines. ISO stands for the International Safety Organization and AHT MIS refers to Surgimedics - Advanced Haemotechnologies' management information systems division.

4.0 Validation Overview

4.1 Organization

The following protocol laid out in section 4.2 will be followed and upon completion a Software Validation Report (SVR) will be written stating the results of the validation effort and the action to be taken following the results of the validation.

4.2 Protocol

The following actions and results are to be completed and documented in the SVR.

System Initialization and Calibration Functions

Action	Result
Power up the unit with a VGA driver and monitor hooked up.	System goes directly to manual mode after testing CPU/memory, PCB, and A/D. VGA monitor will display results of tests.
Hook a pressure source and pressure meter up to the pressure monitor and vary the pressure from 0 to 400 mmHg in 50 mmHg increments.	The pressure reading in manual mode varies with the meter. Record the results in a laboratory test notebook.
Hook a tachometer up to the filter motor and vary the speed from 0 to 1600 rpm in 100 rpm increments.	The filter rpm reading in manual mode varies with the tachometer. Record the results in a laboratory test notebook.
Hook a tachometer up to the pump motor and vary the speed from 0 to 45 rpm in 5 rpm increments.	The pump rpm reading in manual mode varies with the tachometer. Record the results in a laboratory test notebook.
Place a calibration block in the glycerol and hemoglobin sensor head. Run an auto sequence to calibrate the sensor.	The glycerol and hemoglobin sensor should return a sensor ready message on the display.
Remove the calibration block from the glycerol and hemoglobin sensor head. Press Reset, then Stop, then Auto.	The sensor should return a sensor fail message and go back to manual mode.
Run an auto sequence correctly calibrated and wait till the sensor readings for the glycerol level and hemoglobin content are displayed. Then place varying samples of hemoglobin content and glycerol level content in the sensor head.	The glycerol level and hemoglobin level displayed should change. Record the results in a laboratory test notebook.

Normal Functions

Action	Result
Remove power from the unit and start the unit back up.	The display should come up and go to the manual mode.
Press the Out # and Out On/Off keys to test if the pinch valves and shaker are functioning.	The pinch valves should open and close, and the shaker should turn on and off.
Press the Filter and Pump up and down keys to test the setting of the motor rpm.	The settings on the display should change along with the pressing of the keys.
Press Auto with a calibration block loaded in the sensor head.	The display should return a sensor ready message and go to the auto display.
Load a disposable and press Start.	The system should go into a user entry mode.
The user must enter the prompted data with both the barcode scanner and the keyboard.	The system should allow entry from both the barcode and the keyboard. After full entry, the system should go to the auto display.
The end of the sequence is reached.	The printer should print out a report containing all the pertinent data.

Abnormal Functions

Action	Result
During a mock Auto sequence turn the power off and then turn the power back on.	Upon the power being turned back on an error screen should appear telling the user to dispose of the unit and press Stop while a report is printed.
During a mock Auto sequence hook a pressure source and pressure meter up to the pressure monitor. Raise the pressure in the monitor to 500 mmHg.	The unit should fail giving an error screen while a report is printed.
During a mock Auto sequence open the pump door.	The unit should fail giving an error screen while a report is printed.

Software Validation Report

for the
ADVANCED HAEMOTECHNOLOGIES
Thawed Blood Processing System

SECTION CONTENTS PAGE

1.0 Purpose

This document comprises the Software Validation Report (SVR) for the development of the software to be used in the Thawed Blood Processing System (TBPS) manufactured by Surgimedics - Advanced Haemotechnologies (AHT). The purpose of this report is to describe the ability of the software to meet the protocol used to validate the correct function of the software. The following items were tested:

1. System initialization functions.
2. Calibration functions.
3. Normal operation functions in automatic and manual mode.
4. Abnormal operation functions in automatic mode.

The SVR does not contain any design or project requirements. Those are included in the Software Design Description (SDD). The SVR document contains information which AHT considers proprietary and is not to be disseminated without prior written consent from AHT.

2.0 Referenced Documents

The following documents are referenced in other sections of the SVP:

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4.0 Validation Report

4.1 Organization

The protocol laid out in the Software Validation Plan (SVP) was followed and completed. Section 4.2 describes the results of carrying out the protocol and section 4.3 describes what was concluded from the results.

4.2 Protocol and Results

The following actions and results were tested. If the software was able to perform the correct function then the action and result were checked off as a Pass, if the software was unable to perform the correct function then the action and result were checked off as a Fail.

System Initialization and Calibration Functions

Action	Result	Pass/Fail
Power up the unit with a VGA driver and monitor hooked up.	System goes directly to manual mode after testing CPU/memory, PCB, and A/D. VGA monitor will display results of tests.	PASS
Hook a pressure source and pressure meter up to the pressure monitor and vary the pressure from 0 to 400 mmHg in 50 mmHg increments.	The pressure reading in manual mode varies with the meter. Record the results in a laboratory test notebook.	PASS
Hook a tachometer up to the filter motor and vary the speed from 0 to 1600 rpm in 100 rpm increments.	The filter rpm reading in manual mode varies with the tachometer. Record the results in a laboratory test notebook.	PASS
Hook a tachometer up to the pump motor and vary the speed from 0 to 45 rpm in 5 rpm increments.	The pump rpm reading in manual mode varies with the tachometer. Record the results in a laboratory test notebook.	PASS
Place a calibration block in the glycerol and hemoglobin sensor head. Run an auto sequence to calibrate the sensor.	The glycerol and hemoglobin sensor should return a sensor ready message on the display.	PASS
Remove the calibration block from the glycerol and hemoglobin sensor head. Press Reset, then Stop, then Auto.	The sensor should return a sensor fail message and go back to manual mode.	PASS
Run an auto sequence correctly calibrated and wait till the sensor readings for the glycerol level and hemoglobin content are displayed. Then place varying samples of hemoglobin content and glycerol level content in the sensor head.	The glycerol level and hemoglobin level displayed should change. Record the results in a laboratory test notebook.	PASS

Normal Functions

Action	Result	Pass/Fail
Remove power from the unit and start the unit back up.	The display should come up and go to the manual mode.	PASS
Press the Out # and Out On/Off keys to test if the pinch valves and shaker are functioning.	The pinch valves should open and close, and the shaker should turn on and off.	PASS
Press the Filter and Pump up and down keys to test the setting of the motor rpm.	The settings on the display should change along with the pressing of the keys.	PASS
Press Auto with a calibration block loaded in the sensor head.	The display should return a sensor ready message and go to the auto display.	PASS
Load a disposable and press Start.	The system should go into a user entry mode.	PASS
The user must enter the prompted data with both the barcode scanner and the keyboard.	The system should allow entry from both the barcode and the keyboard. After full entry, the system should go to the auto display.	PASS
The end of the sequence is reached.	The printer should print out a report containing all the pertinent data.	PASS

Abnormal Functions

Action	Result	Pass/Fail
During a mock Auto sequence turn the power off and then turn the power back on.	Upon the power being turned back on an error screen should appear telling the user to dispose of the unit and press Stop while a report is printed.	PASS
During a mock Auto sequence hook a pressure source and pressure meter up to the pressure monitor. Raise the pressure in the monitor to 500 mmHg.	The unit should fail giving an error screen while a report is printed.	PASS
During a mock Auto sequence open the pump door.	The unit should fail giving an error screen while a report is printed.	PASS

4.3 Conclusions

The system initialization and calibration functions were tested as laid out in the protocol. It was found that the software will test the CPU/memory, PCB, and A/D at boot up of the system. It was also found that if a VGA driver is hooked up to the computer with a VGA monitor, that the results of these tests will be shown. This will allow for easy diagnosis in the laboratory. It was also found that the pressure monitor correctly read pressure and the results of this test are attached in Appendix A as test number SC97002. It was also found that the filter and pump motor rpm correctly ran and the results of this test are attached in Appendix B as test number SC97003. Therefore, the pressure monitor, filter motor, and pump motor are correctly run and displayed by the software.

The glycerol and hemoglobin sensor were also tested for their ability to calibrate. It was found that the software picked up calibration errors when it was supposed to and calibrated correctly when it was supposed to. It was also found that the sensors correctly read and the software correctly retrieved and displayed the glycerol and hemoglobin readings.

The normal functions were also tested as laid out in the protocol. The software was found to be able to control the actuation of the pinch valves in both automatic and manual modes. The software was also found to correctly control the speeds of both the pump and filter motors. The software was tested for its ability to run an automated sequence and was found to be able to perform the automated sequence without any errors.

The abnormal functions were also tested as laid out in the protocol. The software was found to be able to pick up all errors and display them. All error reports and end of process reports are attached in Appendix C.

Therefore, the software was found to pass all areas tested and was found to function properly within the limits of the protocol.

Appendix A Test SC97002

TEST SC97002 - SBIR Pressure Monitor Test

3/14/97 Pat Cullen

OBJECTIVE: To determine the accuracy of the pressure monitor and the ability of the console software to display the correct pressure. This test follows the protocol as laid out in the SVP.

NOTES: Use Proto1 console with software dated 3/14/97 and Biotek pressure meter.

DATA:

Pressure Meter (mmHg)	Console Pressure (mmHg)
0	0
50	48
99	98
146	144
202	200
255	252
313	310
359	354
402	393

CONCLUSIONS: The pressure sensor is accurate and the software is able to read and display the correct pressure.

Appendix B Test SC97003

TEST SC97003 - SBIR Filter and Pump Motor RPM Test

3/14/97 Pat Cullen

OBJECTIVE: To determine the accuracy of the servo-amps and the ability of the software to interpret, control, and display the speeds of the pump and filter motors.

NOTES: Use Proto1 console with software dated 3/14/97 and Shimpo tachometer (# QL95-003).

DATA:

Filter Motor

Set Point (RPM)	Tachometer (RPM)	Console Display (RPM)
0	0	0
102	103.1	107
205	206.6	208
296	297.0	294
399	400.3	394
503	503.9	490
606	607.0	590
697	697.7	673
800	800.8	775
903	904.0	873
994	994.3	961
1097	1097	1058
1201	1200	1158
1304	1304	1257
1395	1394	1344
1498	1497	1440
1601	1600	1540

Pump Motor

Set Point (RPM)	Tachometer (RPM)	Console Display (RPM)
0	0	0
6	6.5	6
11	11.4	12
16	16.2	17
21	21.2	22
26	26.0	27
31	30.9	32
36	35.8	37
41	40.8	43

CONCLUSIONS: The servo-amps are accurate and the software is able to read, control, and display the correct speeds.

Appendix C - Reports

End of Process Report

EXPIRATION DATE & TIME: 3/19/97 14:52 Z

DEGLYCEROLIZED UNIT NUMBER:NONE
PLASMA HEMOGLOBIN OF DEGLYC. UNIT: 000 mg/dl

GLYCEROL LEVEL OF DEGLYC. UNIT: 00.0

START TIME OF WASH PROC.: 3/18/97 14:52 Z

STOP TIME OF WASH PROC.: 3/18/97 14:52 Z

LOT NUMBER OF 12% SALINE: NONE
LOT NUMBER OF PROCESSING SOL.: NONE
ADDITIVE NAME: NONE
LOT NUMBER OF ADDITIVE: NONE
GLYCEROLIZED UNIT NUMBER: NONE
SISTER DEGLYCEROLIZED UNIT NUMBER: NONE
SISTER GLYCEROLIZED UNIT NUMBER: NONE
CONSOLE SERIAL NUMBER: PROTO 1

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FAX: 281.292.7930

Pump Open Failure Report

GLYCEROLIZED UNIT NUMBER: NONE
DEGLYCEROLIZED UNIT NUMBER:NONE
ERROR AND DISPOSAL OF UNIT DUE TO:
PUMP OPEN

START TIME OF WASH PROC.: 3/18/97 14:44 Z

STOP TIME OF WASH PROC.: 3/18/97 14:44 Z

LOT NUMBER OF 12% SALINE: NONE
LOT NUMBER OF PROCESSING SOL.: NONE
ADDITIVE NAME: NONE
LOT NUMBER OF ADDITIVE: NONE
CONSOLE SERIAL NUMBER: PROTO 1

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THE WOODLANDS, TEXAS, USA 77381
TEL: 800.669.9001
FAX: 281.292.7930

Over-Pressure Failure Report

GLYCEROLIZED UNIT NUMBER: NONE
DEGLYCEROLIZED UNIT NUMBER:NONE
ERROR AND DISPOSAL OF UNIT DUE TO:
OVER-PRESSURE

START TIME OF WASH PROC.: 3/18/97 14:43 Z

STOP TIME OF WASH PROC.: 3/18/97 14:43 Z

LOT NUMBER OF 12% SALINE: NONE
LOT NUMBER OF PROCESSING SOL.: NONE
ADDITIVE NAME: NONE
LOT NUMBER OF ADDITIVE: NONE
CONSOLE SERIAL NUMBER: PROTO 1

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Power Failure Error Report

GLYCEROLIZED UNIT NUMBER: DEGLYCEROLIZED UNI
T NUMBER:ERROR AND DISPOSAL OF UNIT DUE TO:
POWER FAILURE

START TIME OF WASH PROC.: 1/0/70 00:00 Z

STOP TIME OF WASH PROC.: 3/18/97 14:41 Z

LOT NUMBER OF 12% SALINE: LOT NUMBER OF PROC
ESSING SOL.: ADDITIVE NAME: LOT NUMBER OF AD
DITIVE: CONSOLE SERIAL NUMBER: PROTO 1

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Appendix G: Personnel

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	Abdul Balogun
	Patrick Cullen
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	Scott Woodley
	Mike Nichols
	Gary Gage
	Andrew Singer
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DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
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